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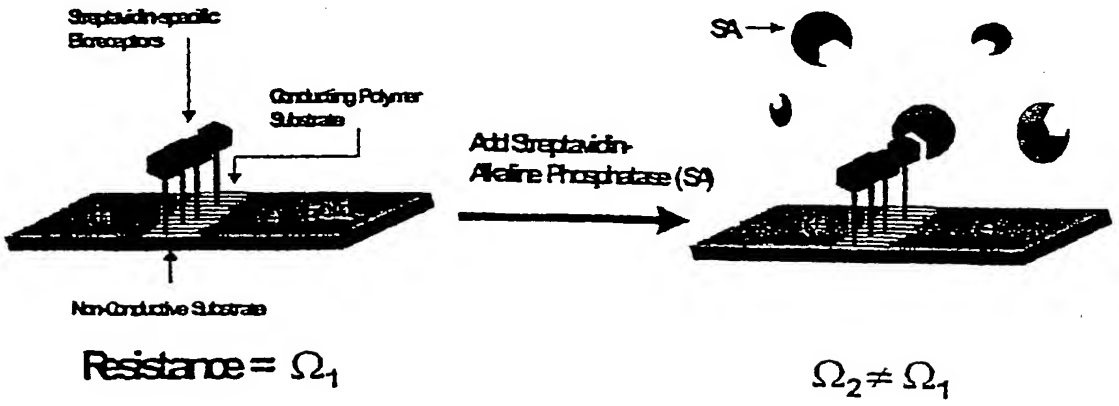

(57) Abstract: Disclosed is an implementation for detecting at least one analyte in a sample. In one embodiment, the implementation includes a sensor array featuring one or a plurality of test sensors. Typically, eachof the test sensors includes a set of electrodes configured with a insulating surface to form a chamber. The implementation further includes a semi-conductive film positioned at least in the chamber; and a polyfunctional linker comprising a first end attached to a receptor and a second end. Also provided are methods for making and using the implementation.

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SENSORS COMPRISING A SEMI-CONDUCTIVE POLYMER

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims benefit to U.S provisional application serial no. 60/240,152 as filed on October 14, 2000. The disclosure of the 60/240,152 provisional application is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention generally relates to implementations for detecting an analyte in a sample. In one aspect, the invention features sensors for detecting a wide spectrum of bioactive analytes including pathogenic cells and particles. Also provided are methods for making and using the implementations. The invention has many important uses including providing biosensors for the rapid detection of amino acid sequences as well as bacterial and viral pathogens and DNA.

BACKGROUND

There has been interest in developing sensors to detect analytes. Analytes of particular interest (sometimes called ligands) have included bioactive molecules, cells, and non-cellular bioactive particles such as viruses. Sensor detection of small molecules such as drugs and environmental contaminants have also attracted much interest. See generally Rogers, K.R. in *Mol. Biotech.*, 14: 109-129; (2000); Laval, J.M in *Analyst*, 125: 29-33 (2000); and Freitag, R. in *Curr. Opin. Biotech.* 4: 71-79 (1993).

One approach for detecting analytes has been to use a receptor (sometimes called a binding partner or ligand) to bind the analyte. A goal is to form a binding complex between the receptor and the analyte. Formation of the complex can be specific in the sense that the receptor only binds the analyte, or it can be less specific, meaning that the receptor can bind other molecules in addition to the analyte. In many but not all instances, the receptor is selected to specifically bind the analyte and form a specific binding complex. Sensor detection of the binding complex is taken to be indicative of presence of the analyte in a sample of interest. Absence of the

binding is taken to mean that the analyte is absent in the sample or present in an amount that is below the sensitivity or selectivity of the sensor.

There have been efforts to develop specific sensors for detecting analytes.

For example, some sensors have been designed to output a detectable signal, usually electronic, when a desired analyte is present in the sample. Such a sensor typically includes electronic components that measure voltage (potentiometric), current (amperometric), light, sound, temperature or mass (piezoelectric) differences produced by formation of the binding complex. See e.g., Lowe, C. R., *Biosensors* 1: 3-16(1985) and Wohltjen, J., *Analytical Chemistry* 56:87-103 (1984).

More specific sensors have been referred to as biosensors, bioaffinity sensors and the like. In particular, there have been reports of enzyme-based and metabolic biosensors. Both types are disclosed as relying on enzymatic or metabolic processes to detect a reaction product. That product often arises through catalytic contact between the enzyme and its substrate.

An example of an enzyme-based biosensor is one that uses glucose oxidase or urease based electrodes. Such enzyme electrodes are known and many are commercially available. See e.g., Vadgana, P., *Journal of Medical Engineering Technology*, 5: 293-298 (1981); Solsky, R.6., *CRC Critical Review of Analytical Chemistry*, 14:1-52 (1983).

See also Taylor, R.F., *The World Biotech Report* 1986, Vol. 2, pp.7-18 (1986) for a discussion of biosensors that are reported to use an immobilized receptor (e.g., an antibody). Analyte binding to the receptor is thought to produce a detectable change in the shape or conformation of the receptor. This change is disclosed as manifesting an output signal that is indicative of the presence of the analyte in the sample. As reported, the change can be optical (interference, refractive index, fluorescence, etc), mechanical (mass or density) or temperature dependent.

See also U.S. Pat. Nos. 5,352,574 and 5,001,048 (disclosing other immobilized receptors for detecting an analyte).

Particular attention has focussed on sensors with antibody-based receptor molecules. See e.g, Aizawa, M., et al., *Journal of Membrane Science* 2:125-132 (1977). In this instance, binding between the receptor and antigen is disclosed as producing a registerable electrical change.

Other biosensors are known. For example, there have been reports of optical biosensors based on antibody-antigen binding. As disclosed, the biosensor works by communicating a change in receptor conformation, the receptor environment, or both. The reported signal changes can be detected and amplified using conventional transducer technology. See Place, J. F., et al., *Biosensors* 1:321-353 (1985).

Others have described efforts to immobilize receptors and particularly enzymes on surfaces such as glass (U.S. Pat. No. 4,357,142), polymer surfaces (U.S. Pat. Nos. 4,352,884; 4,371,612; 5,897,955), and protein films (U.S. Pat. No. 5,001,048).

More recently, biosensors that employ a semi-electroconductive polymer to transduce a signal have been disclosed. For example, see Uchida, I.J. *Electroanal. Chem. Interfacial Electrochem.* 300: 111 (1991); and U.S. Pat. Nos. 5,766,934; 5,312,762 (and references cited therein).

There have been problems associated with many of the prior sensors.

For example, a significant drawback has been the inability of many prior sensors to detect analyte binding in less than a few minutes. This problem negatively impacts sensor performance in many ways. Specifically, such sensors may not always be able to analyze flow stream samples very well. In addition, real-time output to a user may be compromised especially in medical settings in which rapid analyte detection is needed for optimal patient care.

Additionally, many of the prior sensors have been plagued by less- than-adequate signal-to-noise ratios. This has impeded good sensor performance especially when samples having low (trace) amounts of analyte are measured. Samples that have signal interfering substances have been difficult to analyze using such sensors.

Many of the prior sensors also have problems outputting a useful signal to a sensor end user. More specifically, there have been difficulties achieving reliable and effective signal transduction from the receptor-analyte binding complex to an attached conductive polymer. This problem has caused many shortcomings including loss of sensor sensitivity and/or selectivity.

There have been attempts to resolve some of these problems.

For example, there have been reports of sensors in which the receptor (sometimes termed the binding agent) is soaked into or co-polymerized with a semi-conductive polymer. See U.S. Pat. Nos. 4,444,892; and 5,192,507. However, as reported, the relationship between the receptor and the polymer combined in this manner is not believed to be optimal. More particularly, such sensors are not believed to associate the receptor and polymer in a way that provides for effective signal transduction. Implementation sensitivity and user satisfaction are often compromised as a result. Significantly, it is believed that use of such sensors can often mask analyte binding sites, thereby decreasing sensitivity and/or selectivity of the sensor in many instances.

There have been other difficulties associated with many of the prior sensors.

For example, it has been difficult to establish a suitable receptor density in many instances. This drawback has impeded good sensor performance.

For example, sensors with an inadequate receptor density may not always be sensitive or selective enough for particular analyte samples. Such sensors may not always be able to detect analytes reproducibly or with an acceptable cost/benefit ratio.

In addition, many of the prior sensors may not always be able to detect trace analytes or analytes having short lifetimes. In many medical settings, this problem can be especially grave resulting in misdiagnoses and jeopardized patient care.

Sensors having unsuitable receptor densities may especially suffer from low sensitivity and reliability particularly when good analyte binding relies on interaction with more than one receptor.

Significantly, many of the prior sensors are not compatible with miniaturization strategies that are often needed for optimal use. Sensor miniaturization is especially needed for applications involving many diagnostic, commercial, robotic, remediation, research and medical applications.

It would be desirable to have sensors featuring especially good signal transduction between the receptor and the polymer. It would be particularly desirable to have sensors with good contact relationship between the receptor and the polymer to facilitate efficient signal transduction. Further, it would be desirable to have sensors and especially biosensors that can be miniaturized and can output signal to an end-user in less than a few minutes.

SUMMARY OF THE INVENTION

The invention generally relates to an implementation for detecting at least one analyte in a sample as well as methods for making and using the implementation. More specifically, the invention relates to an implementation that provides sensitive and rapid detection of a wide range of analytes such as bioactive molecules, particles and cells; and small molecules. The invention has a variety of important uses including the detection of amino acid sequences as well as cell and viral pathogens such as those implicated in food poisoning, tuberculosis, and pediatric infections.

Particular implementations of this invention feature a sensor array that includes one or a plurality of sensors adapted to detect the analyte (or class of analytes). Each sensor in the array typically includes a set (often less than about ten and usually less than about five or six) of electrodes configured with a suitable

insulating surface to form a chamber. Preferably, a semi-conductive film is positioned in at least the chamber (including, for example, other portions of the sensor or sensor array) sufficient to place the film in electrical contact with the electrodes. As discussed below, it is an object of the invention to place the semi-conductive film in effective contact relationship with at least one analyte binding receptor. Generally, that effective contact relationship is provided by positioning the receptor and the semi-conductive film with a specific linking group as described below.

In embodiments in which the implementation includes more than one analyte binding receptor, each receptor can bind the same or different analyte including a class of analytes. Further, each receptor can be the same as or different from another receptor in the sensor array. Choice of a particular receptor or group of receptors will be generally guided by intended use of the implementation including the analyte (or class of analytes) for which detection is desired.

A preferred semi-conductive film according to the invention is sensitive to analyte binding to the receptor. Preferably, it registers that binding as a change in at least one electrical property of the film. Typically, the change is outputted to an end-user as a detectable signal. That signal is taken to be indicative of the presence of the analyte in the sample. Of course, in cases in which there is no detectable signal, that will often mean that the analyte is not present in the sample.

More specifically, we have found that by providing the good contact relationship between the receptor and the semi-conductive film it is possible to enhance implementation performance in many ways.

For example, the ability of the invention to provide the good contact relationship has been found to improve signal transduction i.e., communication between the receptor and the semi-conductive film. This positively impacts performance of the implementation e.g., by helping to boost sensitivity and selectivity for the analyte. Samples with trace or labile analytes can now be detected efficiently and with an acceptable cost/benefit ratio. Moreover, the good contact relationship helps to minimize implementation response times to less than a few minutes. Such

improved response times facilitates signal output to an end user with less delay and often in real time. This feature improves implementation performance as when the sample is presented as a flow stream or when output results are urgently needed.

The invention generally achieves the effective contact relationship by employing a particular polyfunctional linker between the receptor and the semi-conductive film. By the term "polyfunctional" is meant that the linker can bind more than one binding partner, which partner includes the receptors and semi-conductive films described herein.

A particular polyfunctional linker is configured to join (either covalently or non-covalently) at least one receptor to the semi-conductive film. As will be appreciated, the precise number of receptors joined is usually determined by intended use of the implementation. More preferred polyfunctional linkers suitably space the receptor from the film, thereby helping to optimize binding contact between receptor and analyte.

More specific polyfunctional linkers according to the invention include at least two reactive groups which groups are usually pre-determined to bind (either covalently or non-covalently) the receptor and/or semi-conductive film to the linker. Such preferred polyfunctional linkers often include a "rod-like" component having minimal freedom of movement. An advantageous feature of the complex formed between the receptor, the polyfunctional linker and the semi-conductive film is that the complex serves as an effective conduit for transducing signal from the receptor to the semi-conductive film. That signal can, for example, be electrical, mechanical or a combination thereof. A preferred semi-conductive film is one that can effeciently receive signal from the polyfunctional linker and propagate same (usually at least in part as an electrical signal) towards an appropriate detector and ultimately to the end-user of the implementation.

The good contact relationship provided by the polyfunctional linkers of this invention provide other advantages.

For example, it has been found that when at least the polyfunctional linker is present at a particular density on the film, it is possible to produce an implementation with very favorable performance characteristics. Without wishing to be bound by any theory, it is believed that when the polyfunctional linkers of the invention are suitably close together on the film and with at least a part of the linker in signalling contact with the film, it is possible to transduce signal from the receptor not only vertically (ie. from the receptor to the semi-conductive film) but also horizontally (ie., to another receptor, polyfunctional linker, or both; followed by signal transduction to another part of the film). This feature of the invention can result in a horizontal "domino effect" that helps increase sensitivity to the analyte. Such a domino effect can, in some embodiments, facilitate signal amplification, thereby helping to assist with the detection of many analytes such as those that have short lifetimes or are present in trace quantities.

Sometimes, a suitably dense configuration of polyfunctional linkers will be referred to herein as an aggregate or network of aggregates of polyfunctional linkers.

More specific polyfunctional linkers of the invention include reactive groups usually positioned at at least one end of the linker. In embodiments in which the polyfunctional linker has at least two of such reactive groups, they can be the same but in most cases they will be different from each other. Other formats are within the scope of this invention as when at least one reactive group is attached to the linker between the ends of the linker or attached to the linker by one or more chemical moieties. For most applications however, a first reactive group will be positioned at a first end of the polyfunctional linker, while at the other (second) end, a second reactive group will be placed. Typically, but not exclusively, the first and second reactive groups will be chemically different from one another.

A preferred second reactive group is adapted to provide effective bonding between the polyfunctional linker and the semi-conductive film. Typically, such a preferred group will be chemically reactive. Preferably, it will also be controllably activatable. By the term "controllably activatable", it is meant that one can readily

control the amount and/or extent of reaction between the polyfunctional linker and the semi-conductive film by applying a controllable reaction stimulus.

For example, in invention embodiments in which very dense receptor aggregates (including networks of aggregates) are desired, controllable activation of at least the second chemically reactive group will be highly beneficial. In this instance, such activation allows the user to first generate aggregates or networks having high densities. Such controllable activation also allows the user the freedom to first make multiple networks having two or more different receptors. This feature of the invention provides significant advantages including allowing the manufacture and use of implementations with customized receptor networks having a pre-determined density.

More preferred second chemically reactive groups are generally sensitive to a controllable stimulus that can be initiated e.g., by the implementation user. In this invention embodiment, it is often but not exclusively preferred that the first end of the polyfunctional linker not be as sensitive to that stimulus. Preferably, the first end is essentially insensitive to the stimulus. Particular stimuli of interest are light and temperature. In cases in which the second chemically reactive group is sensitive to light, it is preferred that the group be photoactivatable.

In embodiments in which the second end of the polyfunctional linker is photoactivatable, an especially good contact relationship can be achieved between the receptor and the semi-conductive film. In particular, it has been found that photoactivation of such polyfunctional linkers produces a plurality of covalent bonds that positively impact effective signal transduction. More particularly, such photoactivatable polyfunctional linkers have been found not only to anchor the receptor to the film but also to help form an efficient signal conduit between the receptor and the film.

As discussed, preferred polyfunctional linkers of the invention feature a first chemically reactive group, which group is usually, but not exclusively, positioned on the first linker end and which is often, but not exclusively, insensitive to the stimulus

for activating the second chemically reactive group. The first chemically reactive group is generally flexible in the sense that it can support a range of attachment strategies between the polyfunctional linker and the receptor. Choice of one strategy in lieu of another will be guided by recognized parameters including the receptor and analyte to be detected, the level of sensitivity and/or selectivity desired, and the type or quantity of sample to be analyzed. More preferred first chemically reactive groups will support covalent bonding between the polyfunctional linker and the receptor. Additionally preferred groups will be capable of bonding the receptor to the polyfunctional linker by non-covalent means, specifically by at least one of hydrogen bonds, ionic bonds (e.g, salt bridges), hydrophobic interactions and Van der Waals forces. In one invention embodiment, the first chemically reactive group includes at least one electrophilic group capable of covalently or non-covalently bonding the receptor to the polyfunctional linker.

More specific polyfunctional linkers in accord with the invention are suitably amphiphilic (i.e., have an affinity for water and lipid in the same molecule). In this embodiment, such polyfunctional linkers are well suited for associating with aqueous and non-aqueous environments. For example, such a linker can include a polar head group (typically, but not exclusively, as the first chemically reactive group), an adjustable bridge, and a hydrophobic group or tail (typically, but not exclusively, as the second chemically reactive group). In one embodiment, the polar head group is covalently bound to the hydrophobic tail by the adjustable bridge. In this example of the invention, the polyfunctional linker provides an especially good contact relationship between the receptor (usually in a polar environment) and the semi-conductive film (often in a much less polar environment, usually hydrophobic). Significantly, the bridge provides an effective and adjustable spacing between the receptor and film environments. By the word "adjustable", it is meant that the bridge can be designed as needed to suitably space the receptor from the semi-conductive film. Significantly, the length of the adjustable bridge can be customized to suit an intended use or spectrum of different uses.

Accordingly, and in one aspect, the invention features an implementation for detecting at least one analyte in a sample (including a class of analytes as well).

Preferably, the implementation comprises a sensor array which array includes one or a plurality of operably linked test sensors. Also preferably, each test sensor includes at least one and typically all of the following components:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iii) a polyfunctional linker comprising a first end attached to the receptor (covalently or non-covalently) and a second end attached to the semi-conductive film (covalently or non-covalently).

In a particular embodiment of the foregoing implementation, the semi-conductive film includes at least one polymer material that provides the attachment to the second end of the polyfunctional linker. Preferably, the second end of the linker is pre-selected to provide good contact relationship between the receptor and the semi-conductive film. Methods for selecting such polyfunctional linkers, as well other implementation components such as an appropriate semi-conductive film are described below. In general, such methods use what is referred to below as a standard streptavidin biosensor.

Especially good contact between the receptor and the semi-conductive film is typically manifested between the second end of the polyfunctional linker and the polymer material. In most cases, the second end is covalently bonded to the backbone of the polymer material. However, in some invention embodiments effective contact can include covalent or non-covalent bonding to at least one chemical group (linking moiety) attached to the polymer backbone. As discussed below, such good contact can also be provided when the second end is intercalated within the polymer material or when it is non-covalently associated with the polymer material. As discussed, especially preferred polyfunctional linkers also effectively space the receptor from the film while providing an effective conduit for signal transduction to the film.

Also preferably, the electrode set described above will include electrodes comprising or consisting of a substantially non-corrosive metal or metal alloy. A preferred metal is gold or other suitable metal.

Choice of a particular semi-conductive film to use in accord with the invention will be guided by recognized parameters including intended use. A preferred film is generally conducive to registering analyte binding as a change in at least one electrical property of the film including, but not limited to, resistance, impedance, capacitance, and conductivity. That change is taken by the end-user to be indicative of the presence of the analyte in the sample.

It is an object of this invention to provide flexible implementations that can be customized to suit a desired application or group of applications.

For example, in one embodiment, the implementation includes a detector for receiving and detecting signal passing through the semi-conductive film. That detector is operably linked to at least one of the sensors in the array. Preferably, the detector is adapted to output any change in the electrical property to a user of the implementation. The term "operably linked" is meant to indicate that a given implementation component has a functional relationship with at least one other such component. That functional relationship can be served by integrating the component into the implementation. Alternatively, the component may be in a stand-alone configuration with the implementation.

By way of example and not limitation, the implementation detector can be a "stand-alone" component of the implementation or it can be wholly or partially integrated into the implementation. In embodiments in which miniaturization is desired, integration of the detector and other implementation components will be important and sometimes essential for optimal use of the implementation.

A particular detector for use with the implementation is adapted to receive electrical or light signals outputted by the semi-conductive film. Such detectors are standard in the field and include an ohm meter, capacitance meter and the like.

More specific implementations include a plurality of interdigitated electrodes. In an example, the interdigitated electrodes are each connected to the detector. Preferably, the detector is operably linked to a power supply that can supply the implementation with a power source.

The semi-conductive film can be placed in the implemenetation in one or a variety of ways to help achieve good analyte detection. In one embodiment, the semi-conductive film comprises at least one layer of polymer material, typically between from about 2 to about 10 of such layers. Methods for making such layers including a preferred "layer-by-layer" approach are described below in more detail.

The invention is compatible with use of a variety of different semi-conductive films. Choice of one of the films will be guided by intended use. Also envisioned are blends of individual semi-conductive films (polymer blends). For example, a preferred semi-conductive film will typically exhibit reactivity sufficient to participate in chemical bonding with the polyfunctional linker, usually but not exclusively at the second end. In many embodiments, the polymer material of the film will bond to the polyfunctional linker end covalently although in some examples of the invention non-covalent binding will be preferred.

In more specific embodiments of the invention, a suitable semi-conductive film is further selected to manifest an increase or decrease in electrical conductivity between the electrodes in the presence of the analyte. Alternatively, the film can be selected to manifest an increase or decrease in one or more of capacitance or impedance, in the presence of the analyte.

As mentioned previously, methods for testing performance of a variety of implement components e.g., the semi-conductive film, polyfunctional linker, and receptor, are provided below. A preferred method involves use of a streptavidin-alkaline phosphatase sensor described below in Example 1. As discussed below, one or more components of the streptavidin-alkaline sensor can be replaced with a desired "test" component to evaluate performance in the sensor. Accordingly, the

streptavidin-alkaline sensor can be used to test the functionality of a wide range of semi-conductive films, polyfunctional linkers, and other implementation components to identify those components having desired performance characteristics.

The implementation of the present invention provides other significant advantages.

For example, in preferred embodiments, the implementation provides means for rapidly detecting and identifying analytes often in less than about 2 minutes down to about real time. Generally, the implementation can be assembled at low or moderate cost while providing good sensitivity and selectivity for one or a class of analytes. Significantly, the invention provides essentially reagentless-detection. That is, optimal practice of the invention usually requires no additional materials, such as analyte tags or probes. Also significantly, preferred implementations are easy to use and require low power. In particular embodiments, the implementation is reusable and can be provided with a disposable low-cost sensing element. In embodiments in which the implementation is fully or partially miniaturized, the implementation can serve as a portable, handheld device.

In another aspect, the invention provides methods for detecting an analyte in a sample. In one embodiment, the method includes contacting the sample with an implementation for detecting the analyte or class of analytes. A preferred implementation for practicing the methods includes at least one and preferably all of the following components:

a. a sensor array comprising one or a plurality of test sensors in which each test sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iii) a polyfunctional linker comprising a first end attached to a receptor and a second end.

In a preferred example of the method, the semi-conductive film includes at least one polymer material attached to the second end of the polyfunctional linker. Typically, the film is selected to be conducive to registering analyte binding as a change in at least one electrical property of the film. Preferably, that change is indicative of the presence of the analyte in the sample.

The implementations of this invention can be made by one or a combination of different approaches. For example, in one approach, the implementation can be made by at least one and preferably all of the following steps: layering the semi-conductor film within the chamber, contacting a polyfunctional linker with the film under conditions sufficient to bind the linker to the film surface or within the film; and contacting the receptor to the polyfunctional linker under conditions sufficient to bind the receptor to the polyfunctional linker to make the implementation.

The implementation can be made by other means including at least one and preferably all of the following steps: layering the semi-conductor film within the chamber, contacting a polyfunctional linker with a receptor under conditions sufficient to form a linker-receptor binding complex; and contacting the linker-receptor binding complex to the film under conditions that bind the polyfunctional linker to the film surface or within the film to make the implementation.

In the foregoing methods for making the implementation, conditions sufficient for binding the polyfunctional linker to the semi-conductive film and/or for binding the receptor to the polyfunctional linker generally involve providing reaction conditions conducive to making covalent or non-covalent bonds with the polyfunctional linker. As illustrations, the polyfunctional linker can be covalently bound to the semi-conductive film at one end and the receptor at the other end thereof. However in another embodiment, one end of the polyfunctional linker can be non-covalently bound to the semi-conductive film or be intercalated therein while at the other linker end, the receptor can be covalently or non-covalently bound to the linker. For many invention applications, the receptor will be covalently bound to the polyfunctional linker which linker will preferably be covalently bound to the semi-conductive film.

In more particular embodiments of the foregoing methods, the polyfunctional linker is configured as a thin film prior to or during binding (covalent, non-covalent, or by intercalation) to the semi-conductor film.

In another more particular embodiment, the methods further include making the thin film using a Langmuir-Blodgett device and transferring the thin film from the device to the insulating surface of the implementation. This feature of the invention provides important advantages including providing exceptionally thin and uniform films for use in the implementation. Such thin films have a variety of desirable performance characteristics including the capacity to lend good resistance, capacitance, and/or impedance features to the implementation.

In a particular embodiment of the foregoing methods, the step of layering the semi-conductive film in the chamber can be repeated as needed, usually at least once, to form a multi-layer film having a thickness of from between about 10 Angstroms to about 1000 Angstroms, preferably about 50 Angstroms to about 100 Angstroms. Preferred practice of this method is sometimes referred to herein as a "layer-by-layer" approach.

Other aspects and advantages of the invention are described *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing showing biospecific binding of Streptavidin-Alkaline Phosphatase to a ligand-functionalized conductive polymer-based sensor. Binding of the biomolecule is thought to "twist" the underlying polymer chains and change the electrical resistance of the conductive film.

Figure 2 is a drawing showing exposure of a particular sensor of the invention to a target for which it was intended. A detectable drop in the electrical resistance of the sensor is observed.

Figure 3A-B are drawings showing covalent attachment of densely-packed bioconjugate array and mechanism of signal generation on binding analyte. (3A) Bioconjugates are photochemically attached to conducting polymer substrate. (3B) Conducting polymer is perturbed when analyte binds to ligands (not drawn to scale). Polymer conductivity changes.

Figure 4 is a drawing showing synthesis of a preferred bifunctional amphiphilic bioconjugate (linker).

Figure 5 is a drawing showing manufacture of a Langmuir-Blodgett film. An amphiphile solution is spread out on the water surface (in trough). A moveable barrier compresses the amphiphile monolayer to the "condensed liquid" state.

Figure 6 is a drawing showing a preferred (Schaefer) method for transferring a Langmuir-Blodgett fim to a conducting polymer surface.

Figure 7 is a drawing showing construction of one sensor embodiment. In the drawing, step 2 refers to making an antibody component (see text).

Figures 8A-B are schematic drawings showing (8A) mushroom shaped aggregate of rationally designed linkers and (8B) linkers intercalated into a conductive polymer film for signal amplification.

Figure 9 is a schematic drawing illustrating synthesis of a modified rod-coil structure according to the invention

Figure 10 is a schematic drawing showing formation of amphiphilic nanostructures and intercalation into a conducting polymer.

Figure 11 is a schematic drawing showing covalent bonding of antibody fragments to an intercalated rod-coil nanostructure according to the invention

Figures 12A-B are drawings generally showing use of dendrimers according to the invention. (12 A) Ligand-functionalized dendrimers attach to substrate using multiple photoreactive arms. (12 B) Conducting polymer is perturbed when analyte binds to ligand (not drawn to scale). Polymer conductivity changes.

Figure 13 is an illustration showing a general scheme for dendrimer synthesis

Figure 14 is a drawing showing initial mixed convergent synthesis of surface A (photoactive) and surface B (electrophilic).

Figure 15 is a drawing illustrating synthesis of a bifunctional dendrimer

Figure 16 is a drawing showing conjugation of bifunctional dendrimer to a conducting polymer.

Figure 17 is a drawing showing a complete bioligand linked to a surface of a conducting polymer.

Figure 18 is a graph illustrating swelling of a conductive polymer film by an ingressing species causes a change in the electrical conductivity of the film.

Figures 19A-19D are graphs showing a drop in biosensor resistance in the presence of ligand but not the following interferents: (19A) lysozyme, (19B) bovine serum albumin, (19C) pancreatin, and (19D) trypsin.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to implementations for detecting a substance and more specifically to receptor-based sensors for the identification and quantitation of an analyte (or class of analytes) in a sample of interest. Also provided are methods of making and using the sensors. The sensors of this invention, sometimes called biosensors or bioaffinity sensors, typically include at least one receptor for binding the analyte, at least one polyfunctional linker for binding the receptor, and a semi-conductive film comprising at least one polymer material bound.

As discussed above, it is an object of this invention to provide sensors with good contact relationship between the semi-conductive polymer, polyfunctional linker and receptor. Such a sensor provides many advantages including providing for efficient signal transduction between the receptor and the semi-conductive film and good signal output to the end-user.

More preferred sensors of this invention can be used to detect, identify and quantify an analyte of interest in a wide range of samples e.g, liquid, high-water gel, air, mist, vapor, gas such as air, or emulsion sample. In embodiments in which the sample is gaseous (or partially gaseous), known techniques for transfer of an air sample to a liquid stream can be used to provide a liquid sample for use with the sensor.

By the phrase "class of analytes" is meant ligands having similar chemical or physical structures such that one receptor can bind the ligands either specifically or non-specifically. Such "class" binding is a basis for drug activity and toxicity of many substances. See e.g., U.S. Pat. No. 5,001,048 (disclosing analyte classes e.g., drugs, organophosphorus compounds, viruses, hormones, toxins, and environmental chemicals and pollutants that bind the acetylcholine receptor).

In one sensor of the invention, a pre-selected receptor is bound (covalently or non-covalently) to a suitable polyfunctional linker including those specific bifunctional linkers and dendrimeric linkers disclosed herein. That polyfunctional linker is subsequently bound (covalently or non-covalently) to an appropriate semi-conductive film such as those described below. In embodiments in which the polyfunctional linker is bifunctional, such a linker typically includes what is referred to as a "rod-like" component which component preferably has a height of from between about 0.1nm to about 100nm, preferably about 0.5 nm to about 50 nm. In this example of the invention, the bifunctional linker has a preferred molecular weight of between from about 0.5 kDa to about 100 kDa as determined by gel permeation chromatography or other suitable assay with between about 1 kDa to about 20kDa being preferred for many applications.

As discussed, the sensors of this invention are compatible with a wide variety of suitable bifunctional linkers.

For example, one such linker has one end (terminus) with a photoactivatable group such as an azide group. Such an end preferably can undergo nucleophilic/electrophilic conjugation to or with a desired analyte (ligand molecule). Another preferred linker is between from about 2.0 to about 500.0 nm in length. An additionally preferred linker has an overall shape of a distorted cylinder with a preferred diameter of from about 1nm to about 5 nm. More preferably, the overall length of the linker is less than 50 nm and the van der Waals volume is preferably between from about 60 to about 150 nm^3 as determined by light scattering. Most preferably, the overall length of the linkers would be less than about 50 nm and the linkers would be rigid with a radius of gyration from about 2 to about 10 nm (as determined by light scattering) and the ligands would form a self-assembled monolayer

Additionally preferred linkers of the invention are organic or at least semi-organic and include a plurality of carbon-carbon, carbon-nitrogen or carbon-oxygen bonds. Typical of such linkers are those having a group at one terminal that is preferably, but not exclusively, photoactive such as an azido function, an azo function, a carbonyl group, and imine group, a thio group. Additionally preferred polyfuntional linkers have a group at one end (terminal) that preferably, but not exclusively, acts as an electrophile such as a succinimidyl, carbonyl, or a nucleophile such as an amine, hydroxyl, or a halogen.

More specific bifunctional linkers in accord with the present invention include, generally prior to reaction with the receptor and the semi-conductive film, an electrophilic group on the first end of the linker and a photoactivatable group on the second end of the linker. In one example of such a linker, the receptor is covalently attached to the electrophilic group of the bifunctional linker. Typically, a suitable polymeric material of the semi-conductive film is covalently bound to the photoactivatable group of the bifunctional linker. Typically, but not exclusively, the

electrophilic group of the bifunctional linker is polar and the photoactivatable group is hydrophobic.

It will be apparent that the invention is not limited to particular bifunctional or other linkers having chemically specified first and second ends. Accordingly, the linker can include, prior to reaction with the receptor and the polymeric material of the semi-conductive film, a photoactivatable group on the first end and an electrophilic group on the second end of the linker. Often, but not exclusively, the bifunctional linker is attached to the backbone (ie., main polymer chain) of the polymeric material. However in some invention embodiments it may be more desirable to covalently bind the linker to the backbone through a chemical linking group such as those with a joining hydroxyl, keto, carboxyl, ester, cyano or amino group. Choice of whether to include the linking group will be guided by recognized parameters such as intended use of the sensor and may include therefor other suitable chemical linking groups as needed. See e.g., U.S. Pat. No. 5,766,934 for disclosure about making specific polymer linking groups.

In one embodiment, the photoactivatable group of the bifunctional linker is an optionally substituted azobenzene group. In another invention embodiment, the electrophilic group is an optionally substituted maleimide group. Specifically preferred bifunctional linkers are amphiphilic and generally have water and lipid soluble portions.

By the phrase "optionally substituted" as used herein is meant substitution of hydrogen on a particular chemical group with another substituent with the proviso that such a substitution does not significantly impede the function for which the sensor was intended. Specifically, such substitution should not hinder binding between sensor components e.g., the polyfunctional linker and the receptor, the semi-conductive film and the polyfunctional linker, ect. Examples of such groups include substitution at available positions, typically 1 to 4 or 5 positions, by one or more suitable groups such as those disclosed herein. Suitable groups particularly include halogen such as fluoro, chloro, bromo and iodo; cyano; hydroxyl; nitro; azido; alkanoyl such as a C_{1-6} alkanoyl group such as acyl and the like; carboxamido; alkyl

groups including those groups having 1 to about 6 carbon atoms; alkenyl and alkynyl groups including groups having one or more unsaturated linkages and from 2 to about 6 carbon, or 2, 3, 4, 5 or 6 carbon atoms; alkoxy groups having those having one or more oxygen linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those moieties having one or more thioether linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; alkylsulfinyl groups including those moieties having one or more sulfinyl linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; alkylsulfonyl groups including those moieties having one or more sulfonyl linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; and aminoalkyl groups such as groups having one or more N atoms and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; carbocyclic aryl having 6 or more carbons, particularly phenyl.

More particular bifunctional linkers in accord with this invention can be represented by the following Formula I:

A-B-C

Formula I

in which A is defined as the an optionally substituted maleimide group, B is defined as a spacer having a length of from between about 0.1 Angstrom to about 100 Angstroms, preferably from about 1 to about 50 Angstroms; and C is defined as an optionally substituted azobenzene group. In particular invention embodiments, A is maleimide and C is azobenzene. In a particular invention example, B is further defined as a C_1 to C_{50} alkyl group, alkyl polyol, polyoxyalkyl group, polymethylol, or polyoxyethylene group. A specifically preferred bifunctional linker is shown in Figure 4.

As discussed, in some invention embodiments the polyfunctional linker will be a dendrimer. Preferably, that dendrimer is represented by the following Formula II:

$(P)_X^* (M)_Y$

Formula II

in which P is defined as a dendrimer, X represents an integer of 1 or greater, each M represents a receptor according to the invention, y represents an integer of 1 or greater; and

* indicates that the receptor is associated with the dendrimer either covalently, non-covalently, or a combination thereof. Examples of more preferred dendrimers include those having starburst, dense star, rod-shaped and related configurations suitable for association with the receptors and semi-conductive films described herein have been reported in U.S. Pat. Nos. 5,338,532, 4,694,064; 5,527,524; and 5,788,989; the disclosures of which are incorporated herein by reference.

Additionally preferred dendrimers in accord with the invention have a diameter of from between about 10 Angstroms to about 5000 Angstroms, preferably 100 Angstroms to about 1000 Angstroms.

Also preferred is a dendrimer which, prior to reaction with the receptor and the semi-conductive film, has at least one electrophilic group on a first end of the dendrimer and at least one photoactivatable group on a second end of the dendrimer. In this invention embodiment, the receptor will often, but not exclusively, be non-covalently attached to the electrophilic group on the first end of the dendrimer. Preferably, the photoactivatable second end of the dendrimer is further bound to the polymer material. Such a dendrimer can be bound to the backbone of the polymer material in nearly any acceptable way including covalently or non-covalently with covalent attachment being preferred for most applications.

In invention embodiments in which the dendrimer has at least one electrophilic group, that group is typically polar. Dendrimers with at least one photoactivatable group are especially well suited for use with this invention. In this

illustration of the invention, the photoactivatable group is usually, but not exclusively, hydrophobic.

In one sensor embodiment, an appropriately selected receptor is combined with a suitable polyfunctional linker to form a complex. As discussed, the polyfunctional linker can be eg., a bifunctional or dendrimeric linker. Such a complex can, for example, be immobilized by an appropriate chemical or photochemcial reaction with the semi-conductive film and more specifically with the polymer material of that film. Polymerization of the film polymer material can be effected before, during, or after reaction with the receptor-polyfunctional linker complex with reaction after the polymerization being preferred for many uses of the invention. In addition, such polymerization can be achieved before, during or after reaction of the polyfunctional linker with the film in which case a suitable receptor will be bound to the linker before, during of after binding between the semi-conductive film and the polyfunctional linker. More specific methods for combining these components of the sensor are provided below. Nearly any effective means for performing the polymerization can be used with this invention, particularly those which are optimal for polymerizing materials that include or consist of a suitably conductive epoxy resin, polymer, co-polymer, graft co-polymer, or polymer alloy.

Particular conductive and semi-conductive polymers suitable for use with this invention have attracted much interest. For example, there is recognition that organic polymers, like metals and inorganic semiconductors, can be made to support transport of considerable electrical charge. The earliest studies were performed with polyacetylene, among the simplest of all of the conducting polymers. Since then, conductive polymers such as polyanaline, polythiophene, and polypyrrole have found application as artificial muscles, transparent electrodes, variable-tint "smart" windows, solar cells, light-emitting diodes, electrostatic discharge films, electromagnetic interference shielding, and most notably, as chemical and biological agent sensors.

Conducting polymers are generally characterized by an alternating sequence of single and double bonds along the polymer backbone. At the electronic level, this

alternating bond sequence, or conjugation, is believed to help formation of energy "bands"—molecular orbitals through which electrons can travel, much like in metals. On exposure to chemical or biological species, the relative distribution of electrons within these "bands" can change. Such distribution changes may be manifest as changes in the optical properties (absorbance or fluorescence) or electrochemical properties (oxidation/ reduction potentials) of the conductive polymer. Electrical conductivity changes can also occur. Conductivity in conductive polymer films has largely two components: conductivity due to electrons traveling along the backbone of the individual polymer chains (intrinsic conductivity), and that due to electrons "hopping" between polymer chains (extrinsic conductivity). While a polymer's extrinsic conductivity is generally several orders of magnitude smaller than its intrinsic conductivity, modulation of both types can be exploited in sensing applications.

Without wishing to be bound by any theory, it is believed that many conductive polymer sensors rely on changes in extrinsic conductivity that occur when a conductive film is swollen by small chemical species, for example. As the polymer chains are pushed apart by the ingressing molecules, the electrical resistance changes (Figure 18). The intrinsic conductivity of a polymer film is sensitive to chemical reactions between reactive analytes and individual polymer chains. For example, acids and bases effect increases and decreases in polymer conductivity, respectively. Mechanistically, modifications to the chemical/ electronic structure of the individual polymer chains has a larger effect on overall film conductivity than do changes in the chains' distance from one another.

Although it is believed that mechanical perturbation of individual chains may play a role in helping conductivity changes, it is stressed that such perturbation may not be the only means of generating such changes according to the invention. In particular, conjugated systems are characterized by spatial overlap of the π-orbitals on adjacent repeating units of the polymer. Conjugation along the polymer backbone is effectively "broken" wherever the repeating units are not coplanar and cannot overlap. While conducting polymer chains are reported to be fairly rigid, they are believed to have twists and kinks along their length. There is understanding that the

electrons will travel along the "unbroken" conjugated system until they reach a "break" (twist or kink), at which point they must "hop" to a neighboring chain. The present invention makes use of this phenomenon to detect biologically-active species like bacteria, viruses, etc.

Many of the analytes detected by the implementations of this invention are thought to be too large to diffuse into the conductive polymer film. Without wishing to be bound to any theory, it is believed that such diffusion of small analytes helps to push the polymer chains apart (affecting extrinsic conductivity) or engage in chemical reactions with polymer chains in the bulk of the film (affecting intrinsic conductivity). It is believed that good detection according to the invention is helped by interactions with the analyte and the conductive film surface, and these interactions are mediated by the presence of film surface-bound biospecific ligands. When these ligands bind to the analyte, it is believed that in many instances the individual polymer chains at the conductive film surface are mechanically-perturbed. It is this perturbation that is thought to facilitate measurable changes in the film's electrical resistance, indicating the presence of the analytes of interest.

A wide spectrum of polymer materials can be used in accord with this invention. Such acceptable materials generally include a plurality of conjugated carbon atom bonds eg., an optionally substituted polyanaline, polythiophene, polyacetylene, polypyrrole, polyparaphenylene, or polyparaphenylene vinylene polymer. As an example, polymerization of such species can be initiated chemically, thermally, or photochemically by addition of an appropriate catalyst, initiator or crosslinking agent, e.g, glutaric dialdehdye (glutaraldehyde), heat, or ultraviolet light, to at least the monomer of the intended polymer, , preferably in combination with the polyfunctional linker. Other suitable materials which can be used to polymerize the polymer material will depend e.g., on the compounds selected and can include SPDP, dimethyl suberimidate, disuccinimidyl suberate, bismal bimidonexane and the like.

Choice of a suitable receptor will be guided by ability to bind a particular analyte or group of analytes for which detection in a given sample is desired. As used

herein, the term analyte of interest refers to an individual analyte of interest or a specific class (or type) of analyte bound by the receptor.

In another embodiment of the invention, the polymer material further includes at least one chemical group adapted to join the second end of the polyfunctional linker to the polymer material. Other suitable polymer materials according to the invention include those featuring an electrical conductivity between from about 10^{-16} to about 10^{-3} $ohm^{-1}.cm^{-1}$ and/or a thermal stability of up to about 200°C as determined by thermal gravimetric analysis.

As discussed, the invention is compatible with use of a wide range of semi-conductive films. Included in such films are those having at least one additive or a component for increasing or decreasing electrical conductivity. An exemplary additive is an optionally substituted lower alkyl diamine, preferably hexanediamine. Preferably, the additive is present in the polymer material in an amount that is conducive to acceptable signal transduction and good signal output.

Examples of suitable components for inclusion in the semi-conductive films of this invention include at least one of a metal, metallic alloy, or a carbon material. Preferably, those components are present in an amount that is conducive to acceptable signal transduction and good signal output. Preferred metals include gold, silver, copper, platinum, and nickel. Preferred carbon materials include carbon black, graphite or a carbon nanotube.

The polyfunctional linkers of this invention including those covalently or non-covalently bound to one or more receptors can be configured in the sensor in nearly any suitable arrangement such that the implementation and more specifically the senor, biosensor, or bioaffinity sensor can detect and optionally quantify the analyte for which it was intended. As discussed, sensors having particularly dense linker networks arc often preferred. In this illustration of the invention, each sensor in the array includes a plurality of polyfunctional linkers having a density of between from about $0.1x10^{11}$ to about $25\ x10^{12}$, preferably about $3x10^{11}$ to about $8x10^{12}$ as determined by atomic force microscopy.

In those invention embodiments in which especially dense linker networks are preferred those networks will sometimes be referred to herein as an aggregate. Preferably, each aggregate has a length of between from about 1 nm to about 500 nm, preferably about 5 nm to about 100 nm.

Preferred receptors according to the invention are generally capable of binding at least one analyte (or class of analytes). Typically a receptor of interest will bind about one analyte which binding can be specific or non-specific as needed.

By the term "specific binding" or similar term is meant a molecule disclosed herein which binds another molecule, thereby forming a specific binding pair, but which does not recognize and bind to other molecules as determined by, e.g., Western blotting, ELISA, RIA, gel mobility shift assay, enzyme immunoassay, competitive assays, saturation assays or other suitable protein binding assays known in the field.. By the term "non-specific" binding is meant capacity to bind more than one analyte (or class of analyte) as determined by standard assays such as radioimmunoassay (RIA), Western blot, ELISA test, ect.

See generally Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989), and Harlow and Lane in *Antibodies: A Laboratory Manual,* CSH Publications, N.Y. (1988), for disclosure relating to the methods for detecting specific and non-specific binding.

Illustrative receptors include nucleic acids (DNA, RNA or DNA/RNA hybrids) as well as synthetic or semi-synthetic derivatives thereof (e.g., nucleic acids including radionuclides or sulfur atoms substituted for oxygen in the phosphodiester linkage). Additional receptors of interest include amino acid sequences having D and/or L amino acids including peptides, polypeptides, proteins. Also envisioned are lipids, carbohydrates, lectins, glycoproteins, glycolipids and the like. More specific peptides, polypeptides and proteins of interest include biological or modified derivatives of same including methylated, acetylated, myristylated and phosphorylated protein and polypeptide sequences. Additionally specific proteins

include antibodies as well as antigen-binding fragments thereof such as $f(ab')_2$ and Fab fragments. Also envisioned are enzymes, preferably surface modified enzymes, as well as substrate binding fragments thereof. Also contemplated are nucleic acid receptors that include or consist of catalytic DNA or RNA.

See also U.S. Pat. Nos. 4,562,157; 5,766,934; 5,001,048; 5,192,507; and 5,352,574 for disclosure relating to other suitable receptors including enzymes, polyclonal antibodies, monoclonal antibodies, cell membrane receptors, lectin, antigen, ect.

As mentioned, the invention is compatible with the detection of a wide spectrum of analytes. Such analytes include, but are not limited to, those analytes which are naturally-occuring, synthetic, semi-synthetic or a result of forced evolution.

Particular examples of natural ligands include nucleic acid (RNA, DNA, or RNA/DNA hybrid), peptide, polypeptide, protein, carbohydrate, enzyme substrate, lipid, fungus, glycolipid, glycoprotein, antigen, virus, prion, metazoan cell; or receptor binding fragment thereof. More specific peptide, polypeptide and protein analytes include biological or modified derivatives of same including methylated, acetylated, myristylated and phosphorylated protein and polypeptide sequences.

Examples of forced evolution analytes include recombinant carbohydrates, nucleic acids (RNA, DNA), antibodies, and peptides. Examples of synthetic analytes include proteins, peptides, antibodies, and nucleic acids made whole or in part by the use of an automated or semi-automated device for manufacturing same.

More specific analytes of interest include hormones, vitamins, cytokines such as the interleukins, cytotoxins such as bacterial toxins, blood factors such as Factor X, growth factors such as human growth factor, environmental toxins such as heavy metals and organometallic compounds. Additionally specific analytes include primate parasites and especially bacterial, helminthic (nemotodes, tapeworms, flukes) and protozoan (malaria, leishmaniasis, giardiasis, amebiasis, trypanosomiasis, ect.) cells

including spirochetes. More specific cells of interest are those associated with human disease or discomfort.

Examples of preferred cell analytes include those bacteria associated with food borne diseases (eg., Campylobact. and Salmonella); Legionnella, Tuberculosis, sexually transmitted diseases (STDs such as Chlamydia, and Gonorrhea). Additionally preferred analytes include viruses such as those associated with immune system dysfunction (HIV), childhood illnesses (chicken pox, epstein barr virus), influenza, herpes (including those responsible for genital warts and cold sores). Also preferred are protein analytes such as those associated with allergies including those of animal (domesticated animals such as cats and dogs), fungal and plant origin.

Other exemplary cell and viral analytes have been reported by Sande, MA and Mandell, G.L. in Chemotherapy of Microbial Diseases, *The Pharmacological Basis of Therapeutics*, pp. 1066-1094 MacMillan Publishing Co. (New York) (1985); the disclosure of which is incorporated herein by reference.

As discussed, the sensors of this invention can be used to analyte samples in a variety of forms such as a liquid, gas, vapor, mist or an emulsion. In some embodiments, the sensor can be used in instances in which the liquid is a flow stream. In such an embodiment, the sensors described herein have several applications including use in the monitoring of industrial effluents and quality control analysis.

The implementations of the present invention including specified sensors can be operably linked to one or more additional components which components can be integrated as part of the implementation. Alternatively, or in addition, such components can be present in a stand alone configuration. Selection of a specific implementation format will often be guided by intended use such as whether a sensor is to be used in the field (in which case stand alone component configurations may be suitable) or in a laboratory setting.

For example, in one invention embodiment, the implementation further comprises a pump operably linked to the implementation. In another embodiment, the

implementation further comprises a computational system operably linked to the detector for manipulating the output. In this example, output is preferably stored by the computational system and optionally processed prior to display to the user. Acceptable manipulations include comparing the output to a standard analyte concentration curve and determining the amount of analyte in the sample. Typically, the output is displayed to the user essentially in real-time.

In certain invention embodiments, it may be useful to include a control sensor which sensor can register implementation changes unrelated to analyte binding. As an example, such a control sensor can include essentially the same components of the test sensor with the proviso that the control sensor not include the receptor.

Other preferred implementations include those capable of remote detection of an analyte as well as those suitable for wireless communication system.

In a more specific embodiment, the implementation includes at least one and preferably all of the following components:

a. a sensor array comprising one or a plurality of sensors in which each sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber having dimensions less than about 10 nm x 10 nm,

ii) a semi-conductive film having a thickness of between from about 50 Angstroms to about 100 Angstroms and positioned at least in the chamber, the film providing electrical contact between the electrodes; and either

iii) a bifunctional linker having a length of from between about 2 nm to 10 nm and comprising a first end attached to a protein, nucleic acid; or analyte binding fragment thereof, the linker further comprising a second end, or

iv) a dendrimer having a diameter of from between about 2 Angstroms to about 10 Angstroms and comprising a first

end attached to the protein, nucleic acid; or analyte binding fragment.

In a more preferred implementation, the dendrimer further includes a second end, in which the semi-conductive film includes at least one of an optionally substituted polyanaline, polyparaphenylene, polyacetylene, polyparaphenylene vinylene, or polythiophene polymer. Preferably, the second end of the bifunctional linker or dendrimeric linker is attached to the polymer, the film being capable of registering analyte binding to the protein, nucleic acid; or fragment thereof as a change in electrical conductivity of the film. That change is taken to be indicative of the presence of the analyte in the sample.

As discussed, the present invention features sensors including particular biosensors and bioaffinity sensors (sometimes also called bioaffinity biosensors herein). Thus, in one aspect, the invention discloses bioaffinity biosensors that include at least one type of modified conducting polymer (sometimes called semi-conductive polymer), usually one of such polymers.

To make a specific bioaffinity biosensor according to the invention, a particular conducting polymer (e.g., polyaniline, polypyrrole, polythiophene, etc.) can be deposited as a film into the narrow channel between two electrodes on an insulating substrate, so as to close what would ordinarily be an open electrical circuit. In some embodiments, the electrodes are interdigitated. The conducting polymers are preferably modified with at least one type of biological macromolecular receptor (sometimes called a ligand). As discussed previously, such receptors include but not limited to proteins (including antibodies and antigen-binding fragments thereof; lectins, antigens, cell membrane receptors such as the acetylcholine (AChR) receptor or ligand binding part thereof), nucleic acids and lipids, as well as synthetically-modified materials from both natural and synthetic sources.

A receptor of interest is attached to the polymer films by one or a combination of different approaches. In one approach, the ligands are first coupled to a reactive agent. The linkage connecting the ligand to this agent may be a covalent bond, non-

covalent bond (using through-space hydrogen and/or van der Waals forces), or ionic bond. The same or different chemistries may be used to then attach the ligand-reactive agent conjugate to the surface of the conducting film, or the ligand may simply be blended into the polymer prior to film deposition.

Figure 1 shows binding of a specific biomacromolecular analyte (SA, streptavidin alkaline phosphotase) to a particular ligand-modified conductive polymer film (streptavidin-specific bioreceptor). As shown, specific binding between the SA-peptide and the bioreceptor effects changes in the electrical resistance of the polymer. See also Figure 2 (showing a clear drop in electrical resistance on exposure to the biomolecular target).

The following discussion relates to more specific sensors of the invention.

A. Real-time Sensor For Rapid Diagnosis of Mycobacterium TB

Zoonoses, or infections acquired from animals, affect both animal care workers and experimental animal populations, and are well-recognized risks in research animal facilities. It has been established that Non-Human Primates (NHPs) can be a source of a variety pathogens. For example, infection with Mycobacterium tuberculosis (TB) can result from contact with infectious aerosols produced by experimentally- and naturally-infected animals. TB is a major international human and animal health issue and screening for this disease can be problematic and expensive.

TB infection is defined as a small number of TB bacteria living in a host but not causing illness. This occurs when TB bacteria are inhaled into the tiny air sacs of the lungs. A host with TB infection does not feel ill, has no symptoms, and cannot spread TB bacteria to others. If the host's immune system becomes weak, however, TB infection can develop into TB disease. Individuals with TB infection are at risk of developing TB disease throughout their lifetime unless properly treated.

NHPs that have been bred and maintained in a well-controlled environment have had little opportunity to contract unwanted infections. Other animals coming from sources that are less regulated and less stable have more opportunity to acquire an infection which can subsequently be introduced into the animal care facility. Due to the time and expense involved with breeding NHPs to a point where they are sufficiently mature to act as a model for human disease, many researchers have no choice but to utilize animals from uncontrolled sources, often the wild.

The challenges in the detection of TB relate to not only the aerosol route of infection, but also to the general expense of the process. Detecting the disease in individuals who show clinical signs (because of high titer of the organism) is not a difficult task. However, TB can exist in a carrier state with intermittent shedding of the organism. This situation requires multiple cultures on a given animal to determine whether the animal is infected. With a laboratory culture costing up to fifty dollars per test, the process becomes prohibitively expensive.

The availability of a real-time detector for TB organisms would offer animal care facilities a cost-effective mechanism for protecting the health of animal care workers and the experimental populations in their charge.

Accordingly, the invention features a real-time sensor for Mycobacterium tuberculosis (the causative agent for tuberculosis in non-human primate populations) that exhibits extremely high sensitivity. In order to achivev the level of sensitivity desired, it is an objective of the invention to adapt the ligand-CP transduction method described herein. To this end, an innovative scheme that significantly increases the density of bioconjugates (biospecific ligand + linker arm) attached to the CP film surface is proposed. Preliminary experiments made use of water soluble, photoactive bioconjugates. These bioconjugates were covalently attached to the film by UV irradiation of dilute bioconjugate solutions in which the film was immersed. Using this approach, the maximum ligand surface density achievable was limited by (1) the bioconjugate solution concentration (i.e., the rate at which the bioconjugates diffused to the film surface), (2) the probability that the photoactive termini of the bioconjugates actually attached to the surface (rather than to other surface-bound

ligands), and (3) the steric constraints imposed by the bioconjugate's hydrodynamic volume (the amount of space each surface-attached bioconjugate requires at thermodynamic equilibrium). The proposed approach addresses all three of these limitations simultaneously. First, artificial packing of the bioconjugates together in a prescribed orientation and at high density will be achieved. Subsequently, attachment will be initiated photochemically of the ordered aggregates to the CP film. This is described in detail below.

As mentioned above, the bioconjugate consists of a photoactive base and a bioactive ligand. The base will be processed to form a densely-packed array (i.e., like a thick "lawn") covalently bonded to the surface of the conducting polymer film by simple irradiation with UV light. Following the conjugation of the base to the film surface, electrophilic termini on the base will be covalently bound to the ligand using standard solution chemistry. In this novel approach, it is also possible to pre-form the bioconjugate (including both base and bioactive ligand), then covalently bond it to the surface of the conductive polymer film. The ligand may be an antibody or antigen-binding fragment thereof that will bind irreversibly to a specific analyte. With regard to relative sizes, the base will be approximately 5 A across by 25 A long, and the antibody fragment will be approximately 45 A across. In contrast, the M. tuberculosis bacterium is approximately 0.1 -1 Ilm in diameter. Upon binding, the conformation of the ligand and the base will change, causing a change in the conformation of the surface of the conducting polymer, as we have previously shown. The densely-packed bioconjugate array should afford a 1 to 2 order of magnitude amplification of the signal due to the larger disruption of the conformation of the conductive films. This is schematically represented in Figures 3A and 3B.

B. Real-time Sensor For Rapid Diagnosis of Pediatric S. Pneumoniae and Varicella

The present invention encomapasses a unique approach to sense and detect the specific pathogens responsible for Pediatric S. Pneumoniae and Varicella. In one embodiment, the inventioin provides for real time diagnosis of these diseases and

offers significant cost savings to the health care industry in identifying and treating these conditions. The proposed sensors will obviate the use of time-consuming laboratory tests and will offer the clinician the opportunity to prescribe infection-specific drugs.

In cases in which an infant patient presents with a Fever Without Source (FWS) or Fever of Unknown Origin (FUO), a number of infectious agents such as bacteria or viruses could be the cause. Rapid and specific diagnosis of these pathogens will result in a significant improvement in the quality of care received by the child.

To detect pathogens associated with these diseases, the invention featurs a particular pediatric biosensor. That sensor has many commercial medical applications for point of care diagnosis of pneumonia and chicken pox. It will be appreciated that such a pediatric biosensor can be used for the adult population as well. This basic technology behind the proposed sensors has many applications in detecting and quantifying bacterial and viral pathogens of any kind.

The challenges for the clinician engaged in the diagnosis of the pediatric patient are numerous. In cases in which an infant patient presents with a Fever without source (FWS) or Fever of unknown origin (FUO), rapid and specific diagnostics and therapies would be preferred. However, the required time and physical complexity of obtaining sufficient bodily fluids for culture and identification, and the incubation period required for bacteriological identification of an infectious agent have precipitated the frequent and prophylactic use of broad spectrum antibiotics. Even before a diagnosis is made antibiotics are prescribed. The rational for this approach is commonly that "time is the enemy". Particularly with patients who cannot communicate the specifics of their discomfort or potential exposure, early intervention on a broad scale is preferential to a laboratory-based diagnosis due to the fragility of the patient. The risks with the first exposure of a young patient to a broad spectrum antibiotic are that a drug reaction, allergic reaction, or hypersensitivity may occur. Additionally, if the patient is presenting in an urgent situation, intravenous introduction of antibiotics will likely precede oral introduction and may exacerbate an

adverse response. Overuse of broad spectrum antibiotics has also been implicated in the increased observation of a number of antibiotic resistant organisms.

Time is one of the most critical elements in diagnostic-therapeutic cascade, both in urgent and scheduled medical care situations. In cases where FWO and FWS present, a number of infectious agents such as bacteria or viruses could be the cause. Standard diagnostic protocol dictates the procurement of samples from the patient for bacteriological examination. This examination involves culturing the samples in order to obtain sufficient numbers of infectious agent to allow identification. A prescribed period of time is required for the identification process to be completed. In some cases, two weeks pass before laboratory results are available. In cases that present in the clinic, samples are delivered to the in-house laboratory. However, with samples obtained in the offices of private physicians, a transportation protocol must be followed to get the samples out of the office, to the hospital or commercial lab where the tests are performed.

These problems highlight the urgent need a diagnostic tool that will identify specific infectious agents, both viral and bacterial, in real time, in the clinician's office, without the expense and time delay of a laboratory-based assay. Rapid identification of infectious agents will allow clinicians to tailor specific therapeutic solutions to meet the immediate needs of pediatric patients.

The present invention proposes, in one embodiment, a unique approach to the sensing and detection of the specific pathogens that are responsible for Pediatric S. Pneumoniae and Varicella. This inventive concept allows real time diagnosis of these diseases and offers significant cost savings to the health care industry in identifying and treating these conditions. The proposed sensors will obviate the use of time-consuming laboratory tests and will offer the clinician the opportunity to prescribe infection specific drugs.

More specifically, a more sensitive biosensor is proposed. In a particular embodiment, the biosensor will employ linkers intercalated in the bulk of the CP film to amplify the perturbation registered in the film by the binding event. Development

of a sensor that would be specifically used for identifying and quantifying pediatric pneumonia and chicken pox is an objective of this invention. Of course, the resulting sensor can also be used for the diagnosis of these diseases in the adult population. The details of the proposed concept are described in the following paragraphs.

In the traditional construction of a conducting polymer film-based biosensor, a bioligand is either intimately mixed into the conducting polymer matrix or is tethered to the film surface. The most popular mode of action involves conversion of the target species to some electroactive product that then diffuses into and reacts with the underlying polymer film layer. After reacting with this electroactive species, the polymer exhibits a measureable change in conductivity (i.e., electrical resistance). For example, organo phosphohydrolases (OPH) will hydrolyze agents (such as the insecticide paraoxon, or the nerve agent sarin) to phosphoric acid, which may then be detected by measuring the change in resistance in the conductive polymer film. It is not intuitively obvious, however, that the effect of a simple bioligand/target binding event (which causes a conformational change in the bioligand) could be transferred to the underlying polymer. In fact, it has been demonstrated that the binding of an antibody (attached to a conducting polymer film directly or through a short spacer arm) does not generate a detectable signal due to the rigid structure of the antibody. (That is, minimal conformational change occurs in the antibody on binding). However, preliminary experiments have indicated that using a similarly-rigid bioreceptor system, a significant change in the CP's electrical resistance can indeed be generated. It is believed that the resistnace change generated by a simple binding event between target molecule (or cell) and bioligand (antibody) may be amplified by intercalating the spacer arm into the conducting polymer, rather than just attaching it to the film surface.

To accomplish this, use previously reported techniques to synthesize amphiphilic "rod-coil" triblock macromolecules is proposed. These species consist of a rigid, "rod"-like segment, and a flexible "coiled" segment. As described in the literature, rationally-designed rod-coil macromolecules form a thermodynamically-favored nanostructure in which the rod-like segments self-assemble into a closely packed array, while the coil-like segments segregate into

their own nanophase. The resulting "mushroom" shape and the dimensions of these nanostructures are shown in Figures 8A and 8B. It should be noted that in the same way that the individual rod-coil molecules are amphiphilic, so the aggregated nanostructure is also amphiphilic, with the bundled rod-like structures being hydrophilic and the coil-like segments being hydrophobic (or vice versa).

Amphiphilic molecules tend to align themselves in hydrophobic or hydrophilic media so as to realize the most energetically-favorable orientation. That is, in aqueous Langmuir-Blodgett layer-by-layer film fabrication, for example amphiphilic species orient themselves at the air-water interface so that their hydrophobic ends protrude out of the water, while their hydrophilic ends remain immersed in the aqueous subphase. Similarly, and in accord with this invention, it is proposed that the hydrophilic rod-like stalks of the "mushroom" nanostructures will remain immersed in an aqueous solution of conducting polymer (or polymerizable monomer thereof), while the hydrophobic coil-like "caps" will protrude above the liquid surface into the air. The nanostructure may be self-assembled by dispersing the individual rod-coil macromolecules in cyclohexane, allowing them to aggregate into the "mushroom" structures, then adding the organic phase to the surface of the aqueous polymer solution. The cyclohexane can then be removed in vacuo, after which the aggregates will orient themselves as just described, forming a thin film at the air-water interface. After removal of the water, the rod-like stalks of the "mushrooms" will remain intercalated into the conductive polymer film. The bioligands are then attached to the electrophilic maleimide end group of the coil.

Since the target biologicals in this example of the invention are the causitive bacteria for pediatric pneumonia and the viral agent for chicken pox, which are much larger than the antibody bioligands, the antibodies will bind to these targets at multiple sites on the coil-like "mushroom" segment above the conducting polymer. (The dimensions bacteria and viruses are approximately 1000 to 5000 nm and approximately 10 to 50 nm, respectively). A bacterial cell, in particular, will also not simply bind to one "mushroom" structure, but rather to many. The effect will be to exert force on several individual "mushrooms" at once, each of which will have several attachment points to the underlying conductive polymer (rather than one

single attachment point, like the spacer arms we employed in our preliminary work). The result will be a very pronounced change in the polymer's conductivity. As these coils separate to accommodate the presence of the large analyte species, they will induce a "tilting transition" in the rod-like stalk (embedded in the conducting polymer) to relieve the stress. In the "tilting transition", the individual rod-like portions of the macromolecules within the "mushroom" slide against each other and will cause a conformational change in the conducting polymer. This conformational change will then give rise to a change in the polymer's electrical resistance.

Based on this concept, a biosensor can be designed that is specific to the pathogen in question and can both identify and quantify it. Such a biosensor has many commercial medical applications for point of care diagnosis of pneumonia and chicken pox. Though this effort is geared toward pediatric sensors, the resulting sensors can be used for the adult population as well. This basic technology behind the proposed sensors has many applications in detecting and quantifying bacterial and viral pathogens of any kind.

C. Real-time Sensor For Rapid Diagnosis of Food Poisoning Pathogens

The present invention also contemplates real-time surveillance of foodborne pathogens. This feature of the invention offers significant cost savings to the food processing industry and hospitals in preventing, identifying, and treating food poisoning cases. The proposed sensor will facilitate the monitoring of food facilities and offers a lowcost, real-time alternative to current clinical diagnostic methodologies. Each year, foodborne illness in the United States causes over 20 million cases of human illness resulting in 10,000 annual deaths and a financial impact estimated at tens of billion dollars.

More particularly, the invention provides a unique approach for the detection of specific pathogens responsible for a significant portion of foodborne illnesses (e.g. Salmonella Enteritides and E. coli).

One approach according to the invention relies on providing a good transduction mechanism for detecting biomolecules. The proposed concept builds on

these developments to develop a second generation, inexpensive, yet accurate biosensor that can identify and quantify pathogens in real time. Foodborne illness in the United States is a major cause of personal distress, social disruption, preventable death and avoidable economic burden. As identified and described by the Massachusetts Department of Public Health Working Group on Foodborne Illness Control, a leading agency in epidemiologic investigation, the challenge of managing foodborne pathogenesis is a wide-ranging activity spanning a breadth of disease states and mechanisms of infection. In the late 1990's, foodborne diseases caused an estimated 24 to 81 million sporadic and outbreak-associated cases of human illness and 10,000 deaths annually in the United States. The economic impact of illness at this scale is staggering since the unpleasant symptoms of even a mild case of foodborne illness may require absence from school or work. Some investigators estimate that the financial impact of diarrhea foodborne illnesses is between $7 and $17 billion per year in the United States alone.

The majority of foodborne diseases are caused by microbial pathogens such as viruses, bacteria and parasites. One way of categorizing foodborne illness is by the mechanism by which it is inititated: a) foodborne infection, which occurs when the infectious organism is ingested and invades and multiplies in the victim's intestinal lining, or b) foodborne intoxication, where an organism produces a toxin in food that is subsequently ingested. Ingested pathogens, transmitted from contaminated foods, enter the body by way of the gastrointestinal (GI) tract. The healthy human body has defenses to fight these pathogens, but an overwhelming dose of pathogens or a weakened resistance can lead to illness. Certain populations (for example, the very young, the elderly, and some immunocompromised persons) are at higher risk for foodborne disease and for serious complications of foodborne disease. The severity of illness may be different among people eating the same contaminated food. The variability in illness severity is due to several factors, including: the concentration of the pathogen, the virulence of the pathogen, and the health status of the host.

Currently, approaches to the detection and identification of pathogens that cause foodborne diseases in food production and preparation facilities include both scheduled and unannounced testing for the presence of harmful organisms. This

involves gaining physical access to food production, preparation, inspection, packaging and distribution facilities, and sampling the environment with swabs, open plates and other techniques to obtain samples for cell culture. Once obtained, cultures are plated on a variety of media and allowed to grow. Once there are sufficient numbers of organisms to examine, and the culture has not become contaminated, a biochemical analysis is initiated. The cultures are maintained and observed in some cases up to 14 days. During that time cultured organisms are identified and classified. If during the course of the culture and identification process, a pathogen is discovered a prescribed plan of action is implemented. This forensic approach (on-site sampling + pathogen isolation + 2-week lab culture + identification/ classification) requires a lengthy culture period for the growth of the organism that results in an "unprotected period" between potential pathogen sampling and final bacteriological identification. That is, the time constraints dictated by culture-based diagnostics could result in the release of contaminated food products because in many cases food products are not held in quarantine pending the results of facilities testing.

Once an individual has contracted a foodborne disease, an immediate clinical solution is often demanded. Emergent care includes the evacuation of the GI tract, hydration, sampling for bacteriological identification of the infecting agent, and remediation of the causative agent. As indicated earlier, this process requires significant financial overhead and is painfully slow in the light of clinical urgency.

The traditional culture-based bacteriological analyses have been shown to be a reliable approach for contaminant detection. However, the high cost of maintaining and supplying the laboratories and supporting highly trained staff, in addition to the time required between sampling and pathogen identification suggest that this process could benefit from a real time, organism-specific identification system.

The present invention features a unique approach to the sensing and detection of specific pathogens that are responsible for a significant portion of foodborne illnesses (such as Salmonella enteritidis and Escherichia cold 0157:H7). In one embodiment, the sensor allows real time surveillance of foodborne pathogens and offers significant cost savings to the food processing industry and hospitals in

preventing, identifying, and treating food poisoning cases. This surveillance may take place at various points on the chain of food processing and preparation events, as well as at the site of clinical care for a foodborne illness. The proposed sensor facilitates the monitoring of food facilities and workers and offers a low-cost, real-time alternative to current clinical diagnostic methodologies.

More specifically, the invention features a more sensitive biosensor to detect these cells. One proposed approach uses dendrimer technology to amplify the perturbation registered in the CP film by the binding event. It is specifically proposed to make a sensor that would be specifically used for identifying and quantifying food poisoning pathogens.

Preliminary data demonstrate that the binding of a large biomolecule to a suitable bioligand will change the electrical resistance of the conductive polymer film to which the ligand is attached. This scheme obviates the need for generation of a secondary electroactive moiety that must diffuse into the conducting polymer to elicit an effect, as in enzyme-based glucose sensors. In order to amplify the direct-transduction effect, the invention features use of highly-branched dendrimers as ligand linkers. As described previously, dendrimers have well-defined architectures, and thereby can covalently attach to the conductive polymer film at many different points, causing greater mechanical perturbation of the film when the analyte binds to the ligand. The nascent construction of dendrimers will permit the synthesis of structures that contain two distinct surfaces. One surface will be activated to covalently bond to the conducting polymer surface and the other, when coupled to the bioligand, will interact with food poisoning bacteria. The surface that will bond with the conducting polymer surface will be photoactive. The surface that will bind to the target cell will use a prebonded IgG antibody fragment against surface epitopes of the target.

Bacterial cells are relatively large (0.2 to 0.6 ,um in diameter), while the dendrimers are smaller nanostructures that may have diameters of approximately 0.01 to 0.1 ,um. Consequently, a bacterial cell will not simply bind to one dendritic macromolecule, but rather to five or ten. The cell will then exert force on several

individual dendrimers at once, each of which have several attachment points to the underlying conductive polymer (rather than having only one single attachment point, like the linkers we used in our preliminary experiments). This is shown schematically in Figures 12A and 1B. The result will be a very pronounced change in the polymer's conductivity.

It will be apparent that the proposed biosensor has applications in monitoring food manufacturing lines as well as point of use diagnosis of pathogens responsible for foodborne diseases. This integrated system has dual use applications as a medical diagnostic tool (e.g., for screening biopsied tissue samples), or for maintaining supermarket/restaurant/ cafeteria quality control (e.g., spoilage/contamination assessment).

As discussed, the invention provides specific biosensor for detecting a peptide that binds streptavidin. In one example of such a sensor, the peptide is combined with an (*N*-hydroxysulfosuccimidyl-4-azidobenzoate) linker which linker is combined with a suitable semi-conductive film such as polypyrole under conditions that activate the azidobenzoate group to effect covalent bonding thereto. Generally, but not exclusively prior to such photoactivation, the film was subjective to known conditions suitable for polymerizing the film. In most cases, such polymerization is achieved by mixing and casting monomer between the set of electrodes, generally within a few minutes after initiating polymerization. The result is a semi-conductive film. As discussed througout this application, such a film can be may "layer-by-layer" which has been found to produce exceptionally sensitive sensors. If desired, the coated semi-conductive film can be aged for a sufficient time, usually about a few hours up to a day or so.

The streptavidin sensor just discussed (sometimes also called a streptavidin-alkaline sensor) is described more specifically in Example 1 below. Such a sensor can be used to test the functionality of nearly any sensor component including a desired semi-conductive film, receptor and polyfunctional linker. Particular combinations of such films, receptors and linkers can also be conveniently tested. Importantly, such a sensor can be used to select or modify those components

(receptor, polyfunctional linker, polymer) having one or a group of desired performance characteristics.

Testing a polyfunctional linker. Referring now to Example 1 below, the (*N*-hydroxysulfosuccimidyl-4-azidobenzoate) linker of the standard streptavidin sensor can be replaced with a test polyfunctional linker preferably also having the azidobenzoate end group. The test linker can then be covalently bound to the peptide as described. This conjugate can be added to the surface of a polypyrrole (or other suitable conducting polymer as described herein) film and subjected to UV irradiation to induce covalent bonding to the film. After the ligand-functionalized film equilibrated somewhat in glass-distilled deionized water, the film can be exposed to a solution of Streptavidin-Alkaline Phosphatase (SA). The test polyfunctional linker is "acceptable" if it facilitates at least about a 20% decrease in the resistancepreferably at least a 50% decrease and more preferably from about 75% to about 95% or more of the resistance decrease seen with the standard streptavidin biosensor.

Testing other sensor components. It will be apparent that other components of the foregoing streptavidin biosensor can be used to test other components such as the type of conductive film including films having two or more conductive polymers. Also, the addition of various polymer additives and components can be evaluated by using this biosensor. A preferred conductive film, additive, or film component is acceptable if it facilitates at least about a 20% decrease in the resistance, preferably at least a 50% decrease and more preferably from about 75% to about 95% or more of the resistance decrease seen with the standard streptavidin biosensor.

A variety of receptors suitable for use with this invention including particular antibodies and cells can be obtained from the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, VA 20110-2209.

The present invention will now be illustrated by the following examples. The examples are not intended to limit the scope of the invention in any way.

Example 1: Biosensor For Detecting Streptavidin binding Peptide

A proprietary peptide which binds tightly to Streptavidin-Alkaline Phosphatase was linked to the reactive agent Sulfo-HSAB (*N*-hydroxysulfosuccimidyl-4-azidobenzoate). The Sulfo-HSAB moiety on the resulting conjugate had a photo-reactive azide group. This conjugate was added to the surface of a polypyrrole (conducting polymer) film and subjected to UV irradiation to induce covalent bonding to the film. After the ligand-functionalized film equilibrated somewhat in glass-distilled deionized water, the film was exposed to a solution of Streptavidin-Alkaline Phosphatase (SA). The result was an irreversible decrease in the resistance of the polypyrrole film (Figure 2). It may be that the binding event encourages closer packing of the individual polypyrrole chains on the surface of the polymer film. This would make it easier for current to flow from one electrode to the other through the film, and would be manifest as a decrease in electrical resistance. (With other receptor /analyte /conducting polymer systems, resistance *increases* are possible as an indication of analyte detection.).

The foregoing peptide can be obtained from Bachem Biosciences Inc. (3132 Kashiwa Street, Torrence CA 90505) as product name CS-373, lot no. B01877. The peptide has the following sequence:

H-Ala-Glu-Gly-Pro-Cys-His-Pro-Gln-Phe-Pro-Arg-Cys-Glu-Gly-Gly-Gly-Ser-Lys- Ala-$NHNH_2$ (SEQ ID No. 1).

The peptide ligand will bind to streptavidin under physiologic conditions (e.g., phosphate buffered saline, pH 7.2) with a dissociation constant (Kd) of about 200nM. The ligand has two primary amines, the alpha amine and the epsilon amine on the lysine side chain at position 18. There is also a hydrazide group at the C-terminus; this group will react with aldehydes to form a stable covalent bond.

Further evidence that such binding had occurred was afforded first by subsequent exposure of the ligand-modified polypyrrole film to *p*-nitrophenyl phosphate. The aqueous solution above the polymer-coated electrodes became yellow in color (due to the generation of *p*-nitrophenolate ion), indicating that the peptide-bound enzyme Alkaline Phosphatase was catalyzing *p*-nitrophenyl phosphate

hydrolysis. (In the absence of this enzyme, no yellow color is observed.) Second, glycerol had been added to the original SA solution to minimize SA aggregation. When the polypyrrole film was exposed to a neat glycerol solution, however, there was no further change in resistance, confirming that the original resistance change was truly due to a ligand-SA binding event. Exposing the sensor to targets for which it was not specific provided further confirmation.

The following specific methods were used to construct the sensor.

1. Synthesis of the Linker-Ligand Conjugate.

To a solution of the Dyax Inc., (Cambridge), peptide, CS-373 [4.2 mg in 300 μL of 10 mM potassium phosphate buffer, (pH 7.12)], was added 400 μL of Sulfo-HSAB, (Pierce Chemical), [2 X 10^{-6} moles of protein and 4.4 X 10^{-6} moles of Sulfo-HSAB]. The reaction proceeded at room temperature for one hour. The solution was used as is for the photo-crosslinking reaction.

2. Photochemical Covalent Bonding of the Linker-Ligand to the Conductive Polymer.

The solution of the Linker-Ligand was added directly to the surface of the cassette of conducting polypyrrole polymer. The cassette was placed on ice and irradiated in a Rayonette photolysis apparatus with a uv maximum at 254 nm for 15 minutes. The cassettes were washed with 30 mL of 10 mM potassium phosphate buffer, (pH 7.12), then with 2 X 30 mL of Deionized, Glass Distilled water, (17.0 ohm resistance). The cassettes were dried overnight then used without further treatment.

3. Binding of Strepavidin to the Linker-Ligand Modifier Cassette.

Commercially available Strepavidin from Pierce, 1 mg/mL in Phosphate Buffer was solvent exchanged with Deionized, Glass Distilled water, (17.0 ohm resistance), using a 10,000 MW cut-off Amicon Centricon. To the surface of the cassette 30 μL of the resulting strepavidin solution, (1 mg/mL), was added and the conductivity was measured.

The sensor of this Example is sometimes referred to herein as a "standard streptavidin biosensor". As discussed above, the sensor can be used to test one or a

combination of sensor components including, but not limited to, a receptor, polyfunctional linker, and semi-conductive film (with or without additives or polymer components).

Figures 19A-D show results of the sensor in the presence of various interferants. (19A) lysozyme, (19B) bovine serum albumin, (19C) pancreatin, (19D) trypsin. In each instance, the sensor showed a significant drop in resistance after adding the SA (streptavidin alkaline phosphatase) reagent.

Example 2- Proposed Biosensor for Detecting *M. tuberculosis* Bacterium

A. Synthesis of Amphiphilic Ligands and Film Formation

For the densely-packed array of ligands we have chosen to use a unique variation on the formation of Langmuir-Blodgett films, using the Schaefer modification method of film transfer. The basic concept is to construct a Langmuir-Blodgett-style film (in a trough) based on the the amphiphilicity of the base linkers at the air-water interface (see Figure 18). The Langmuir-Blodgett film will actually be a monolayer of amphiphilic derivatized ligands that self-assembles using the polar head group of the amphiphile to orient the hydrophilic end of the molecule in the aqueous subphase while the hydrophobic portion of the molecule will remain above the interface. This is the concept of floating a drop of oil on the surface of water as originally described by Benjamin Franklin when he used a layer of oil to induce a calming influence over the water in the Clapham ponds2 . In this process water will be the subphase and the amphiphile will be a 1 -maleimidyl- 1 8-[p-azophenyl] octadecane . This bifunctional reactive am phi phile [as well as other members of its family], while not commercially available, is readily synthesized from the Friedel-Crafts alkylation of 1-[N-maleimidyl]-18-chlorooctadecane and azobenzene with AlCl3. The synthesis is shown in Figure 4.

The trough will be based on the design of Lin et al.3 and will not be discussed further. Once the amphiphile is placed on the surface the trough will compress the two dimensional surface to a point where the area per molecule is approximately 22 A^2. This occurs when the surface pressure is measured to be greater than approximately

20 dyn/cm. Under these conditions the surface is referred to as a "solid phase" or liquid condensed stated. This defines the Langmuir-Blodgett film and is shown in Figure 5. The Langmuir-Blodgett film now has the polar head groups in the aqueous subphase and the alkyl tails densely-packed and held in position by hydrophobic interactions. It should be noted that the bifunctional nature of the amphiphile has not been altered. The polar head group is susceptible to nucieophilic attack and the azo group on the hydrophobic tail is photoactive.

B. Formation and Transfer of the Langmuir-Blodgett linker film

The next step in this scheme is to covalently bond the thick "lawn" of linkers to the conducting polymer. To effect the Langmuir-Blodgett bioconjugate film transfer to the conducting polymer substrate, we propose to use the Schaefer method. In the Schaefer method, the substrate (with polypyrrole already deposited onto it, for example) is placed on top of the Langmuir-Blodgett film. A film of this type (i.e., based on stearic acid) has been accurately measured to extend 25 A above the surface of the subphase. Therefore, placement of the substrate to the contact point of the surface of the Langmuir-Blodgett film may be accomplished with great facility. Under the standard Schaefer method, the film is then lifted free of the subphase, thereby forming a thin layer on the substrates. One of the obvious difficulties with this methodology is freeing the film from the subphase without disruption due to gravity, surface tension and van der Weals interactions. To circumvent this issue we propose the following to ensure that the film has the structural integrity to survive transfer. Upon irradiation with a UV lamp, the azo moiety on the hydrophobic tail will form a nitrene group, which will insert into carbon-hydrogèn bonds of the polypyrrole film, affording a covalent bond between the linker film and the conductive film When the substrate is then lifted from the water's surface, the covalently-bonded, densely packed linker film will remain attached to the substrate. The linker film will provide the high concentration of binding sites for subsequent attachment of biospecific ligands needed for signal amplification. This sequence is depicted in Figure 6.

C. Attachment of the Bioligand to the Linker

With the conducting polymer and densely-packed linker films stitched together, the next step is to covalently bond the ligand to the linker. To capture the

mycobacterium we propose to use a commercially-available antibody specific for the agent. The antibody, which is an IgG molecule found in the immune system, may be rendered chemically-active by reducing the disulfide bonds connecting the two halves of the IgG. These fragments of IgG have been commercially exploited by Pierce Chemical and others due to their extremely tight binding constants, approaching those of the "irreversible" biotin-streptavidin conjugate, Ka= $10^{15}M^{-1}$,[7] (Ka = association constant). This is the basic principle used in antibody affinity chromatography. The IgG may be reduced with mercaptoethylamine to the corresponding sulfhydryl in situ the free sulfhydryl of the antibody fragment will then add in a nucleophilic sense to the maleimidyl terminus of the Langmuir-Blodgett linker film. This concept is demonstrated in Figure 7.

D. Testing of the Biosensor

The sensor, thus constructed, will bind tightly and specifically to Mycobacterium tuberculosis. Given that the mycobacterium is two orders of magnitude larger than any one single ligand, it is likely that several ligands will bind to it at once. The binding events v fill mechanically perturb the dense self-assembled linker nanostructure. Since the linker "lawn" is covalently-bonded to the surface of the conducting polymer (e.g., polypyrrole), the conductive film will also be perturbed, and its conductivity will be altered. In this way, the direct binding of the mycobacterium to the ligand will be converted to an electrical signal via the underlying conductive polymer. The signal derived from the presence of a single mycobacterium will be significantly enhanced due to the binding of the pathogen with many tens of ligands, each of which will contribute to the electrical resistance change. The sensor will be exposed to the M. tuberculosis in aqueous form, and the conductivity measured as a function of analyte concentration. Interferants will include non specific bacteria as well as other biological fluids such as blood, urine and sputum. Titration of the mycobacterium will be done to determine the sensitivity of the biosensor.

With this technology, it will be possible to construct an array of biosensors that will individually alarm for each of the causative agents for food poisoning. The array responses could be used to identify each species of potentially pathogenic

organism present and quantitatively assess the potential risk from contamination. It is thus feasible to construct a complete sensor that would include the sensor array, all the electronics, the display, and a fully-developed logic.

Example 3- Proposed Biosensor For Detecting Pediatric *S. Pneumoniae* and *Varicella*

A. Synthesis of rod-coil structures with electophilic coil termini

The basic synthesis of the rod-coil macromolecule will be accomplished using the techniques pioneered by Stupp and co-workers . The rod-coil assembly strategy is to independently synthesize the rod-like portion of the macromolecule, compound 3 in Figure 9, by a condensation reaction between two stiff units, compounds 1 and 2. The length of the rod like molecule may be controlled to some degree by the choice of starting materials. However, it is critically important that this rigid segment not exceed a certain maximum length, or else it will assume a helical conformation and cease to be purely linear. Should this segment become non-linear, its ability to "pack" in an orderly array. For the synthesis of the coil-like segment, a polyisoprene unit is synthesized using anionic polymerizations. The molecule that initiates the anionic polymerization will be a derivatized isoprene fitted with an electrophilic maleimide for later covalent attachment of the antibody fragment (Figure 9, compound 4). The final condensation of the rod-like segment with the polymer coil leads to the rod-coil structure, compound 5. The molecular weight of these macromolecules is reported to be between 8 and 10 kDa, as determined by gel permeation chromatography against polystyrene molecular weight standards. The polydispersity of these molecules ranges from 1.03 to 1.13, and the volume fraction for the rod segment is approximately 0.2. NMR as well as TOF mass spectrometry in addition to molecular weight determination will be used to characterize these macromolecules.

B. Formation of the amphiphilic nanostructures and intercalation into the conducting polymer

In order to facilitate the self-assembly of the rod-coils into the mushroom-like structures, thin films of the macromolecules are cast from dilute solutions of cyclohexane onto carbon coated glass slides. The rod-like portion of the rod-coil is

soluble in the organic medium. After the solutions have stood unstirred for 24 hours, the solvent is removed under vacuum. The resulting ultra-thin films of the self-assembled mushroom-like nanostructures are floated off of the glass slide using an aqueous solution of the conducting polymer. The aqueous solution at this point consists of the conducting polymer solubilized in the aqueous phase, and the self-assembled rod-like segments intercalated into that phase. The coil-like portions of the rod-coil structures remain above the air/water interface. The aqueous medium is cast onto bare gold-on-glass slide substrates, dried, and annealed. Characterization of this surface following the annealing step will be accomplished using atomic force microscopy, as well with transmission electron microscopy7. In practice, we expect to find a densely packed array of "mushroom-caps", with the aggregated self-assembled coil segments on the surface of the conducting polymer, and the aggregated self-assembled rod segments intercalated into the conducting polymer matrix. The overall features of these nanostructures are shown in Figure 10.

C. Covalent bonding of the antibody fragment to the electrophilic coil terminus

With the formation of the basic sensor platform completed, the next step is to covalently bond the bioligand to the surface of the "mushroom" nanostructure. The strategy is to attach an antibody fragment specific to either of the target biomolecules, S. pneumoniae or Varicella. Antibodies for these causative agents will be fragmented in situ using standard techniques8, leaving an active sulfhydryl function on the antibody fragment. These active fragments will add directly to the maleimide moieties at the termini of the mushroom coil segments. To demonstrate that the technique will work, fluorescent microscopy will be used to quantify the number of fluorescent antibody probe fragments present. The ability of the antibody to bind to the cells and viral particles of interest will be demonstrated by using specific sandwich assays, similar to the motif of an Enzyme-Linked Immunosorbent Assay (ELISA). In the ELISA technique, the cells or viral particles will be bound to the conjugated antibody fragments and secondary antibodies will be incorporated that will have specific reporter functions. The readout may be fluoresence, radioactivity, or an enzyme assay. This will allow us to quantify the number of binding events possible on the surface of the biosensor. The covalent bonding is shown in Figure 11.

D. Testing of the real-time pediatric biosensor

The final test of the biosensor will be to expose the surface of the conducting polymer/intercalated rod-coil/bioligand to the target organism. The system will be a success if the binding event causes a change in conductivity. As negative controls, the system will be challenged with a variety of biofluids and/or simulants, as well as with organisms not specific for the antibody in use. Titration of the biosensor with the target bacterium and viral particle will determine the sensitivity of the instrument. It is expected that having multiple "mushroom"/film attachment sites and multiple antibody/bacterium binding events will result in an amplified detection signal.

Example 4- Proposed Biosensor for Detecting Food Poisoning Pathogens

A. Synthesis of Dendrimers with Bifunctional Surfaces

Most syntheses of dendrimers evolve from the repetitious alternation of growth reactions and activation reactions. The scope and details of these reactions have been reviewed elsewhere'. There are four methodologies that are incorporated into the synthesis of dendrimers, namely: divergent growth, convergent growth, growth using hypercores with branched monomers, and double exponential and mixed growth. It is this latter protocol that we will utilize to develop the bifunctional surfaces. During the growth phase, and generally by the fourth generation, the so-called "starburst limit" has been reached and the surface is so crowded that no further growth chemistry can occur. The basic chemistry generally involves simple addition/substitution reactions, as shown in Figure 13. These units will then be the core elements for the double exponential mixed growth protocol. The general scheme for the growth of the first surface [A] is shown in Figure 14. This nanostructure is a dendron to which new branching moieties may be added to generate the second surface, [B]. This is demonstrated in Figure 15. In our scheme, surface A will contain a hydrophobic arm terminating in a photoactive azobenzene group. Surface B will contain a relatively-polar electrophilic maleimide. Surface A will ultimately bond covalently to the conducting polymer, while surface B will be covalently bonded to the bioligand specific for the target bacteria.

B. Self Assembly and Attachment of the Dendrimer to the Surface of the Conducting Polymer

Due to the bipolar nature of their surfaces, the dendrimers will have the tendency to orient themselves based on the polarity of the processing solvents used. We propose to disperse the dendrimer in an aqueous medium. A thin dendrimer film will form at the air-water interface, with the hydrophobic surface oriented out of the water, and the hydrophilic surface facing down into the water. Once the film has been formed, the conducting polymer-coated substrate will be turned upside-down, with its surface parallel to that of the water, and lowered until it just comes into contact with the dendrimer film at the water's surface. The system will then be irradiated with UV light. As a result, the azobenzene-functionalized hydrophobic termini of the dendrimer will covalently bond to the surface of the conducting polymer, immobilizing the dendrimer. When the substrate is then raised from the water, the hydrophilic dendrimer's surface B. which had been oriented into the aqueous subphase, will be expressed on the outermost film surface, and will be available to participate in subsequent ligand-attachment chemistry (Figure 16). Similar approaches have been previously reported.

C. Covalent Bonding of the Bioligand to Surface B of the Dendrimer

With the dendrimer now immobilized on the surface of the conducting polymer, the next step is the introduction of the bioligand. Each bioligand we have chosen is a fragment of the IgG antibody that is specific to one of the five pathogens of interest mentioned above. These fragments of IgG have been commercially exploited by Pierce Chemical and others due to their extremely tight binding, with binding costants approaching that of the "irreversible" biotin-streptavidin conjugate, $K_a = 10^{15} M^{-1}$, (Ka= association constant).

Binding of this kind is widely used in antibody affinity chromatography. The antibodies used here may be rendered chemically-active by reducing the disulfide bonds connecting the two halves of the IgG. Specifically, the IgG may be reduced with mercaptoethylamine to the corresponding sulfhydryl in situ 5, and the free sulfhydryl of the antibody fragment will then add in a nucleophilic sense to the

maleimidyl terminus of the B surface of the dendrimer. This concept is demonstrated in Figure 17.

D. Testing of the Biosensor

With the dense coating of the bioactive dendrimer on the surface of the conducting polymer, exposure of the conductive film to the target organism should lead to a binding event that will significantly perturb the film's surface, resulting in a measureable conductivity change. The system will be challenged with a variety of biofluids, as well as with irrelevant organisms (targets for which the dendrimer-bound ligands are not specific) as negative controls. Titration of the biosensor with the target organism will be used to determine the sensitivity of the sensor. It is expected that having multiple dendrimer/ film attachment sites and multiple antibody/ bacterium binding events will result in an amplified detection signal.

With this technology, it will be possible to construct an array of biosensors that will individually alarm for each of the causative agents for foodborne illness. The array responses could be used to identify each species of potentially pathogenic organism present and quantitatively assess the potential risk from contamination. It is also feasible to construct a complete sensor that would include the sensor array, all the electronics, the display, and a fully-developed logic. The sensor will be able to accurately and selectively identify and quantify the pathogens causing foodborne illnesses in real time.

All references disclosed herein are incorporated by reference including the following specific references:

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What is claimed is:

1. An implementation for detecting at least one analyte in a sample, the implementation comprising:

a. a sensor array comprising one or a plurality of test sensors in which each test sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iii) a polyfunctional linker comprising a first end attached to a receptor and a second end,

wherein, the semi-conductive film comprises at least one polymer material attached to the second end of the polyfunctional linker, the film being conducive to registering analyte binding as a change in at least one electrical property of the film, wherein the change is indicative of the presence of the analyte in the sample.

2. The implementation of claim 1, wherein the implementation further comprises a detector operably linked to at least one of the sensors in the array, the detector being adapted to output any change in the electrical property to a user of the implementation.

3. The implementation of claim 2, wherein the detector is an ohm or capacitance vector.

4. The implementation of claim 1, wherein the sensor array comprises a plurality of interdigitated electrodes.

5.The implementation of claim 4, wherein the interdigitated electrodes are each connected to the detector.

6.The implementation of claim 2, wherein the detector is operably linked to a power supply.

7.The implementation of claim 1, wherein the semi-conductive film comprises at least one layer of polymer material.

8. The implementation of claim 1, wherein the semi-conductive film provides an increase or decrease in electrical conductivity between the electrodes in the presence of the analyte.

9.The implementation of claim 1, wherein the semi-conductive film provides an increase or decrease in capacitance or impedance between the electrodes in the presence of the analyte.

10.The implementation of claim 1, wherein each sensor in the array comprises a plurality of polyfunctional linkers having a density of between from about $3x10^{11}$ to about $8x10^{12}$ as determined by atomic force microscopy.

11.The implementation of claim 10, wherein the plurality of polyfunctional linkers are configured as an aggregate.

12.The implementation of claim 11, wherein each aggregate has a length of between from about 5 nm to about 100 nm.

13. The implementation of claim 1, wherein the polyfunctional linker is bifunctional.

14. The implementation of claim 13, wherein the bifunctional linker comprises a rod-like component.

15.The implementation of claim 14, wherein the rod-like component of the bifunctional linker has a height of between from about 0.5 nm to about 50 nm.

16.The implementation of claim 13, wherein the bifunctional linker has a molecular weight of between from about 1 kDa to about 20kDa as determined by gel permeation chromatography.

17.The implementation of claim 14, wherein the rod-like component has a volume of between from about 60 nm^3 to about 150 nm^3 as determined by light scattering.

18.The implementation of claim 14, wherein the rod-like component has a radius of gyration of between from about 1 nm to about 100 nm as determined by light scattering.

19.The implementation of claim 13, wherein the bifunctional linker comprises, prior to reaction with the receptor and the semi-conductive film, an electrophilic group on the first end and a photoactivatable group on the second end.

20. The implementation of claim 19, wherein the receptor is covalently attached to the electrophilic group of the bifunctional linker.

21. The implementation of claim 19, wherein the polymeric material is covalently linked to the photoactivatable group of the bifunctional linker.

22.The implementation of claim 19, wherein the electrophilic group is polar and the photoactivatable group is hydrophobic.

23.The implementation of claim 13, wherein the bifunctional linker comprises, prior to reaction with the receptor and semi-conductive film, a photoactivatable group on the first end and an electrophilic group on the second end.

24.The implementation of claim 21, wherein linker is attached to the backbone of the polymeric material.

25.The implementation of claim 19, wherein the photoactivatable group is an optionally substituted azobenzene group.

26.The implementation of claim 19, wherein the electrophilic group is an optionally substituted maleimide group.

27.The implementation of claim 13, wherein the bifunctional linker is from about 0.5 nm to about 50 nm in length.

28.The implementation of claim 13, wherein the bifunctional linker is water soluble.

29. The implementation of claim 19, wherein the bifunctional linker is represented by the following formula:

A-B-C

wherein,

A is defined as the an optionally substituted maleimide group,

B is defined as a spacer having a length of from between about 1 Angstrom to about 50 Angstroms; and

C is defined as an optionally substituted azobenzene group.

30. The implementation of claim 29, wherein B is further defined as a C_1 to C_{50} alkyl group, alkyl polyol, polyoxyalkyl group, polymethylol, or polyoxyethylene group.

31. The implementation of claim 1, wherein the polyfunctional linker and the receptor is represented by the following formula:

wherein P is defined as a dendrimer, X represents an integer of 1 or greater, each M represents the receptor, y represents an integer of 1 or greater; and

* indicates that the receptor is associated with the dendrimer.

32.The implementation of claim 31, wherein the dendrimer has a diameter of from between about 100 Anstroms to about 1000 Angstroms.

33.The implementation of claim 31, wherein the dendrimer, prior to reaction with the receptor and the semi-conductive film, has at least one electrophilic group on a first end and at least one photoactivatable group on a second end of the dendrimer.

34. The implementation of claim 33, wherein the receptor is non-covalently attached to the electrophilic group on the first end of the dendrimer.

35.The implementation of claim 33, wherein the photoactivatable second end of the dendrimer is further bound to the polymer material.

36.The implementation of claim 35, wherein the dendrimer is further bound to the backbone of the polymer material.

37.The implementation of claim 33, wherein the electrophilic group is polar and the photoactivatable group is hydrophilic.

38. The implementation of claim 1, wherein the polyfunctional linker is intercalated within the polymeric material.

39.The implementation of claim 1, wherein the polymeric material comprises a plurality of conjugated carbon atom bonds.

40.The implementation of claim 1, wherein the polymer material further comprises at least one chemical group adapted to join the second end of the polyfunctional linker to the polymer material.

41.The implementation of claim 1, wherein the polymeric material has an electrical conductivity between from about 10^{-16} to about 10^{-3} $ohm^{-1}.cm^{-1}$.

42.The implementation of claim 1, wherein the polymeric material has a thermal stability of up to about 200°C as determined by thermal gravimetric analysis.

43.The implementation of claim 1, wherein the polymeric material comprises a conductive epoxy resin.

44.The implementation of claim 1, wherein the polymeric material comprises a polymer, co-polymer, graft co-polymer, or polymer alloy.

45.The implementation of claim 39, wherein the polymeric material is an optionally substituted polyaniline, polythiophene, polypyrrole, polyparaphenylene, or polyparaphenylene vinglene.

46.The implementation of claim 1, wherein the semi-conductive film further comprises at least one additive or a component for increasing or decreasing electrical conductivity.

47.The implementation of claim 46, wherein the additive is an optionally substituted lower alkyl diamine.

48.The implementation of claim 46, wherein the component is at least one of a metal, metallic alloy, or a carbon.

51.The implementation of claim 48, wherein the metal is gold, silver, copper, platinum, or nickel.

52.The implementation of claim 48, wherein the carbon material is carbon black, graphite or carbon nanotubes.

53. The implementation of claim 47, wherein the lower alkyl diamine is hexanediamine.

54.The implementation of claim 1, wherein the receptor is capable of binding at least one analyte.

55.The implementation of claim 1, wherein the receptor is a nucleic acid or a derivative thereof.

56. The implementation of claim 1, wherein the receptor is a peptide, polypeptide, protein, lipid or a glycolipid.

57.The implementation of claim 56, wherein the protein is a surface modified enzyme; or a substrate binding fragment thereof.

58.The implementation of claim 56, wherein the protein is an antibody; or an antigen binding fragment thereof.

59.The implementation of claim 55, wherein the nucleic acid is RNA or a derivative thereof.

60.The implementation of claim 59, wherein the nucleic acid is catalytic DNA or RNA.

61.The implementation of claim 54, wherein the analyte is one of a nucleic acid, peptide, polypeptide, protein, enzyme substrate, lipid, fungus, glycolipid, antigen, virus, prion, metazoan cell; or receptor binding fragment thereof.

62. The implementation of claim 61, wherein the analyte is a bacterial or protozoan cell.

63.The implementation of claim 1, wherein the sample comprising the analyte is a liquid, gas, vapor, mist or an emulsion.

64.The implementation of claim 63, wherein the liquid is a flow stream.

65. The implementation of claim 64, wherein the implementation further comprises a pump operably linked to the implementation.

66. The implementation of claim 1, wherein the implementation further comprises a control sensor, the control sensor comprising essentially the same components of the test sensor with the proviso that the control sensor not include the receptor.

67. The implementation of claim 2, wherein the implementation further comprises a computational system operably linked to the detector for manipulating the output.

68. The implementation of claim 67, wherein the output is stored by the computational system and optionally processed prior to display to the user.

69. The implementation of claim 67, wherein the manipulation comprises comparing the output to a standard analyte concentration curve and determining the amount of analyte in the sample.

70. The implementation of claim 2, wherein the output is displayed to the user essentially in real-time.

71.The implementation of claim 1, wherein the implementation is capable of remote detection of an analyte.

72. The implementation of claim 1, wherein the implementation further comprises a wireless communication system.

73. An implementation for detecting an analyte in a sample, the implementation comprising:

a. a sensor array comprising one or a plurality of sensors in which each sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber having dimensions less than about 10000 nm x 10000 nm,

ii) a semi-conductive film having a thickness of between from about 50 Angstroms to about 1000 Angstroms and positioned at least in the chamber, the film providing electrical contact between the electrodes; and either

iii) a bifunctional linker having a length of from between about 2 nm to 10 nm and comprising a first end attached to a protein, nucleic acid; or analyte binding fragment thereof, the linker further comprising a second end, or

iv) a dendrimer having a diameter of from between about 2 Angstroms to about 100 Angstroms and comprising a first end attached to the protein, nucleic acid; or analyte binding fragment, the dendrimer further comprising a second end,

wherein, the semi-conductive film comprises at least one of an optionally substituted polyaniline, polyparaphenylene, polyparaphenylene vinylene, or polythiophene polymer in which the second end of the bifunctional linker or dendrimer is attached to the polymer, the film being capable of registering analyte binding to the protein, nucleic acid; or fragment thereof as a change in electrical conductivity of the film, wherein the change is indicative of the presence of the analyte in the sample.

74. A method for detecting an analyte in a sample, the method comprising contacting the sample with an implementation for detecting the analyte, the implementation comprising:

a. a sensor array comprising one or a plurality of test sensors in which each test sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iv) a polyfunctional linker comprising a first end attached to a receptor and a second end,

wherein, the semi-conductive film comprises at least one polymer material attached to the second end of the polyfunctional linker, the film being conducive to registering analyte binding as a change in at least one electrical property of the film, wherein the change is indicative of the presence of the analyte in the sample.

75. A method for making the implementation of claim 1, the method comprising the steps of:

a. layering the semi-conductor film within the chamber,

b. contacting a polyfunctional linker with the film under conditions sufficient to bind the linker to the film surface or within the film; and

c. contacting the receptor to the polyfunctional linker under conditions sufficient to bind the receptor to the polyfunctional linker to make the implementation.

76. A method for making the implementation of claim 1, the method comprising the steps of:

a. layering the semi-conductor film within the chamber,

b. contacting a polyfunctional linker with a receptor under conditions sufficient to form a linker-receptor binding complex; and

c. contacting the linker-receptor binding complex to the film under conditions that bind the polyfunctional linker end of the linker-receptor binding complex to the film surface or within the film to make the implementation.

77.The method of claim 75 or 76, wherein the method further comprises repeating step a) at least once to form a multi-layer film having a thickness of from between about 50 Angstoms to about 1000 Angstroms.

78. The method of claim 75 or 76, wherein the polyfunctional linker is configured as a thin film prior to or during binding to the semi-conductor film.

79.The method of claim 75 or 76, wherein the method further comprises making the thin film using a Langmuir-Blodgett device and transferring the thin film from the device to the insulating surface of the implementation.

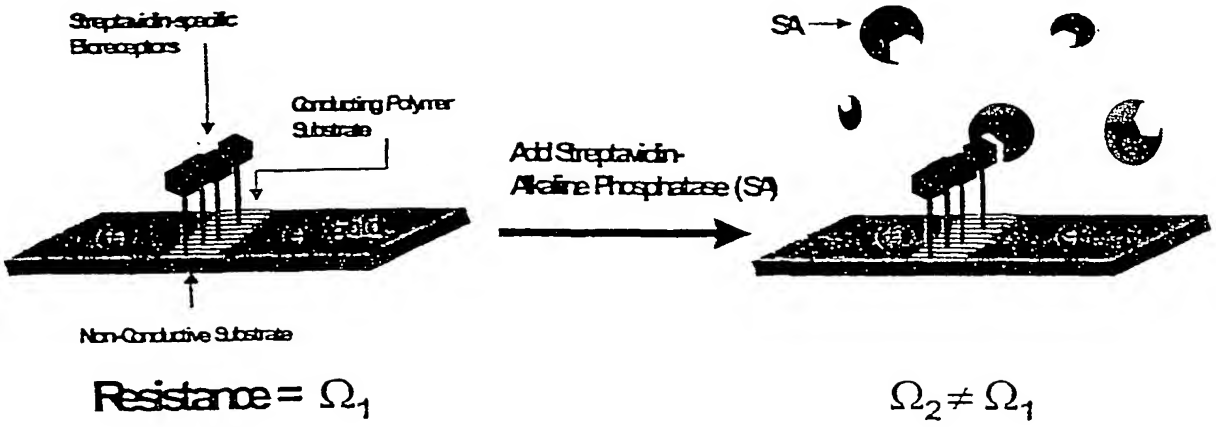


Figure 1

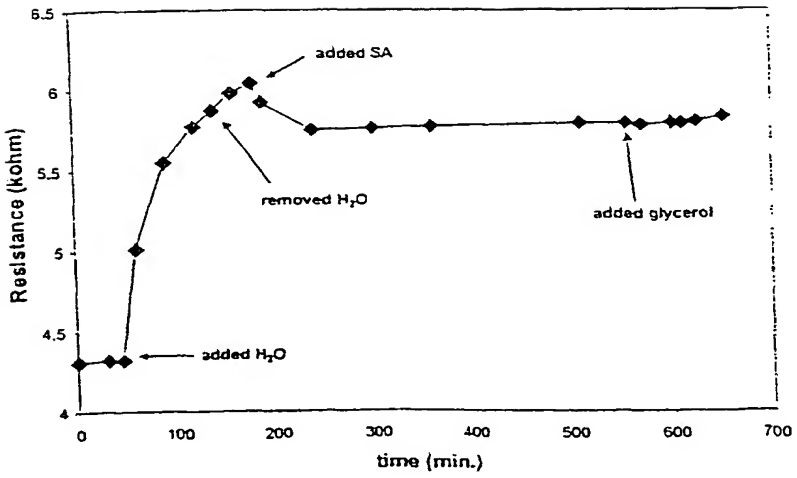


Figure 2

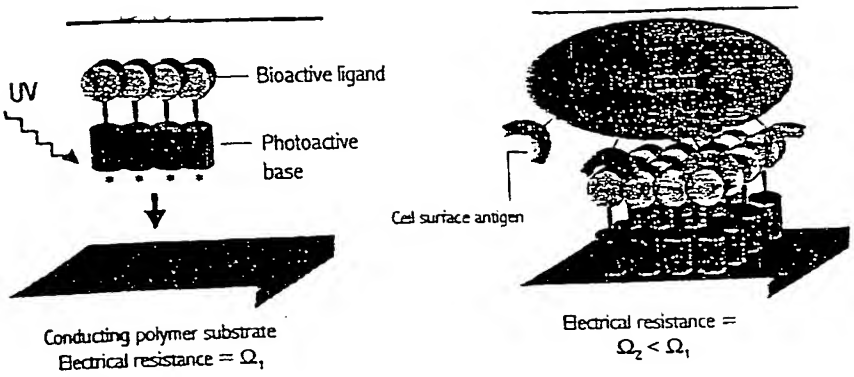


Figure 3

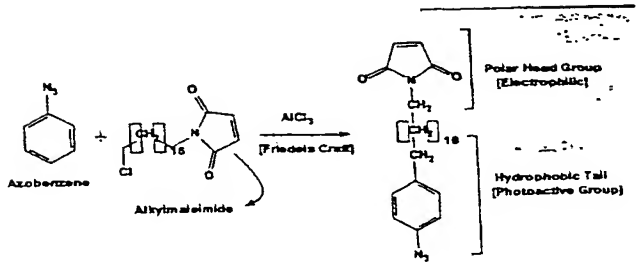


Figure 4

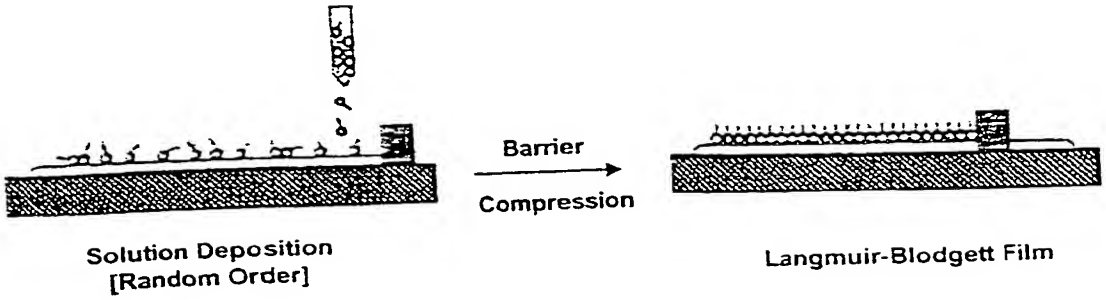


Figure 5

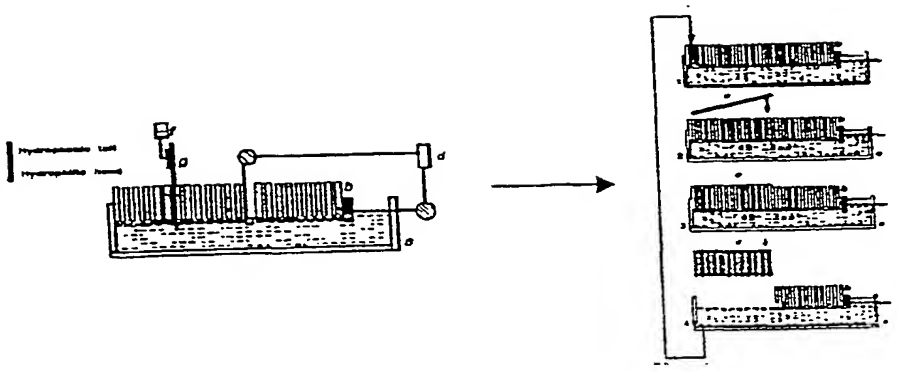


Figure 6

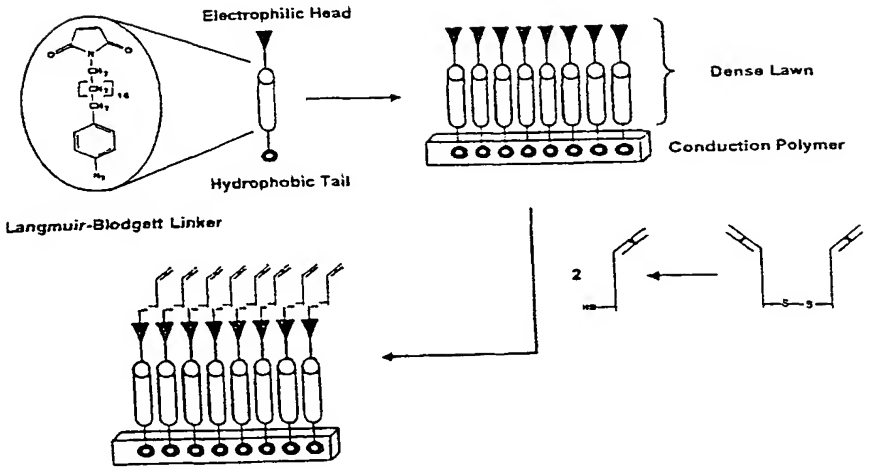
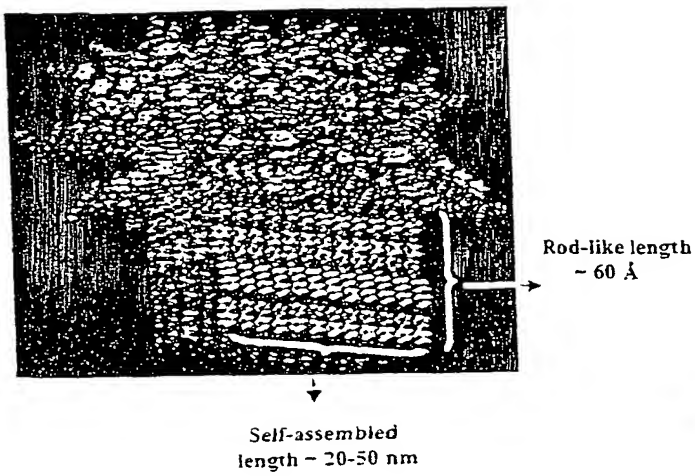
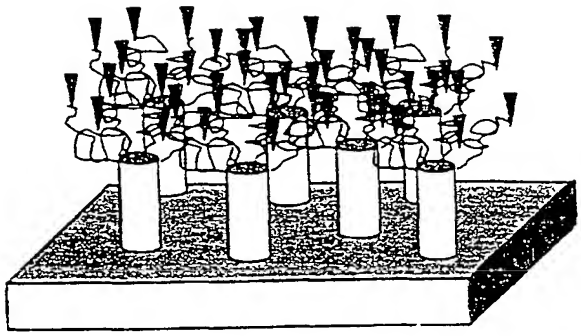


Figure 7



(a)

Fig. 8A



(b)

Fig. 8B

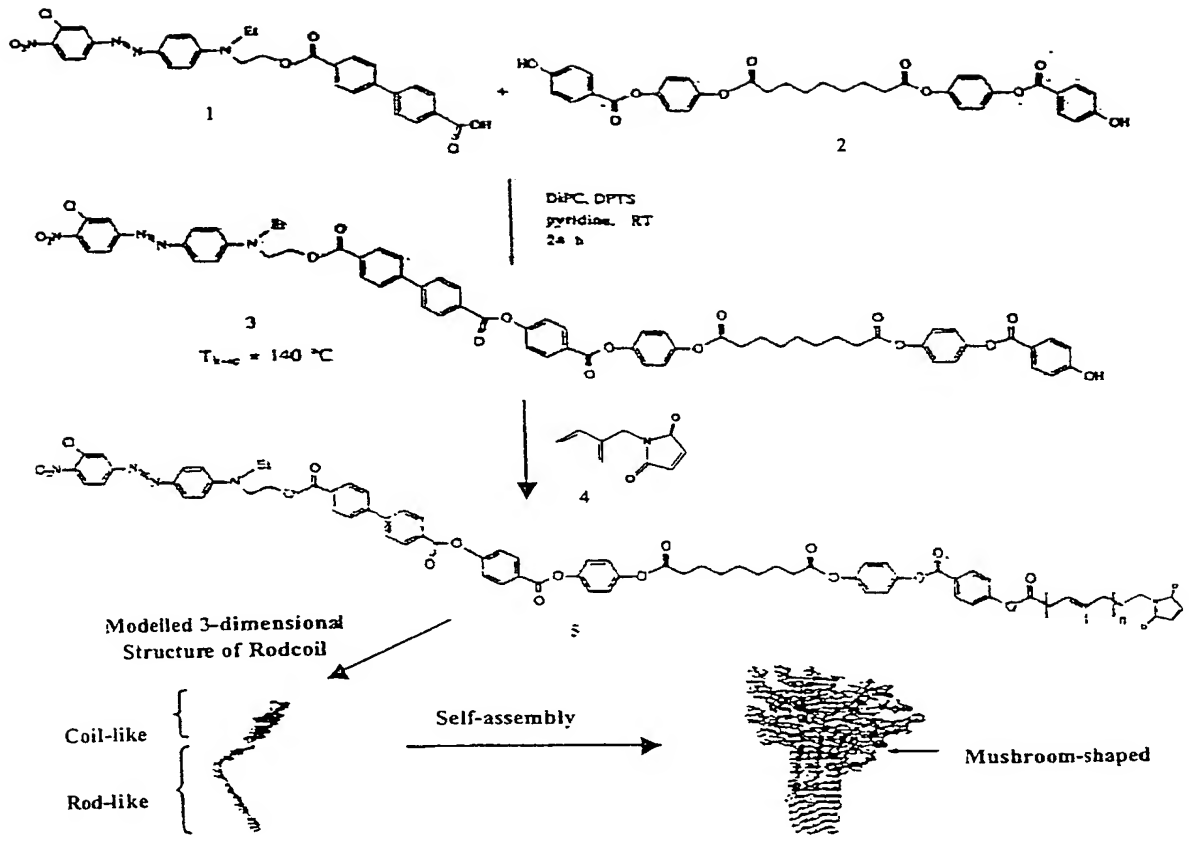


Figure 9

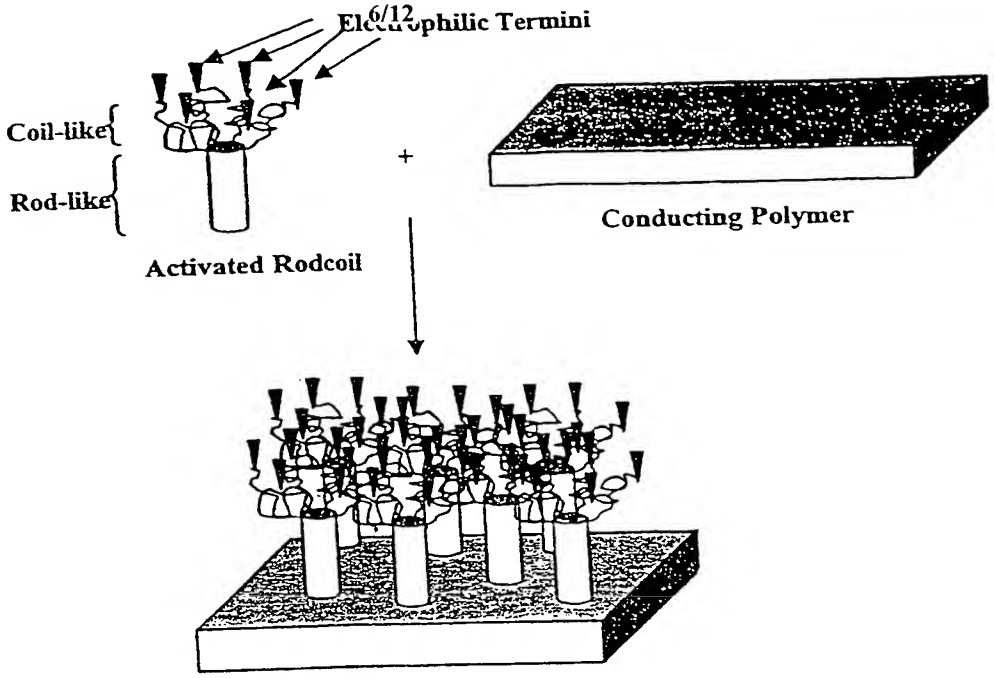


Figure 10

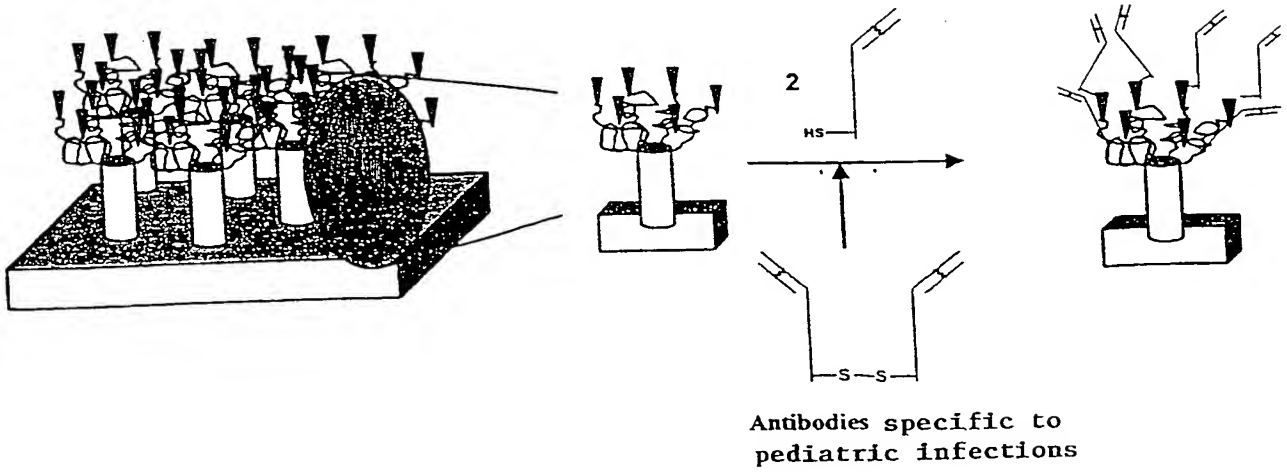


Figure 11

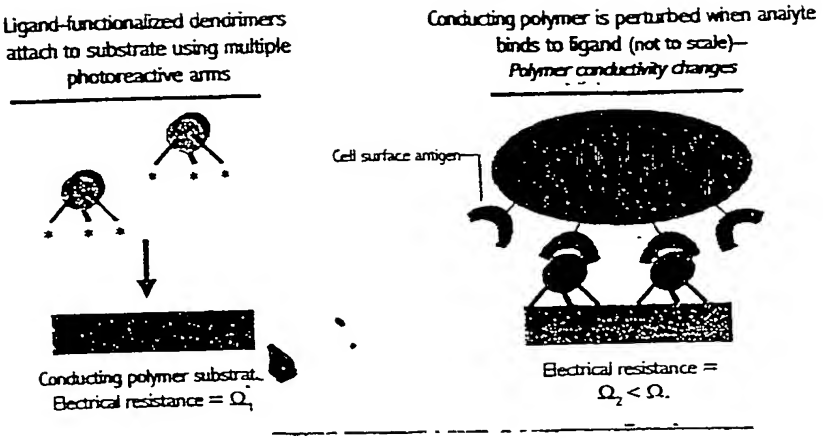


Figure 12

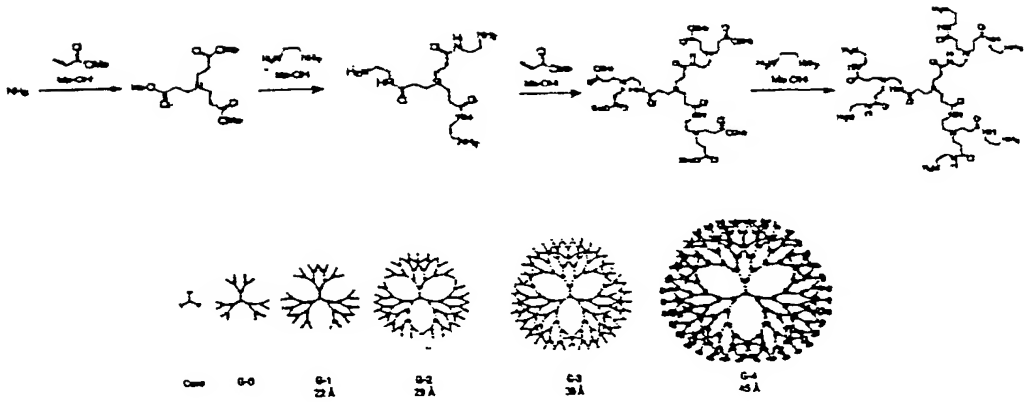


Figure 13

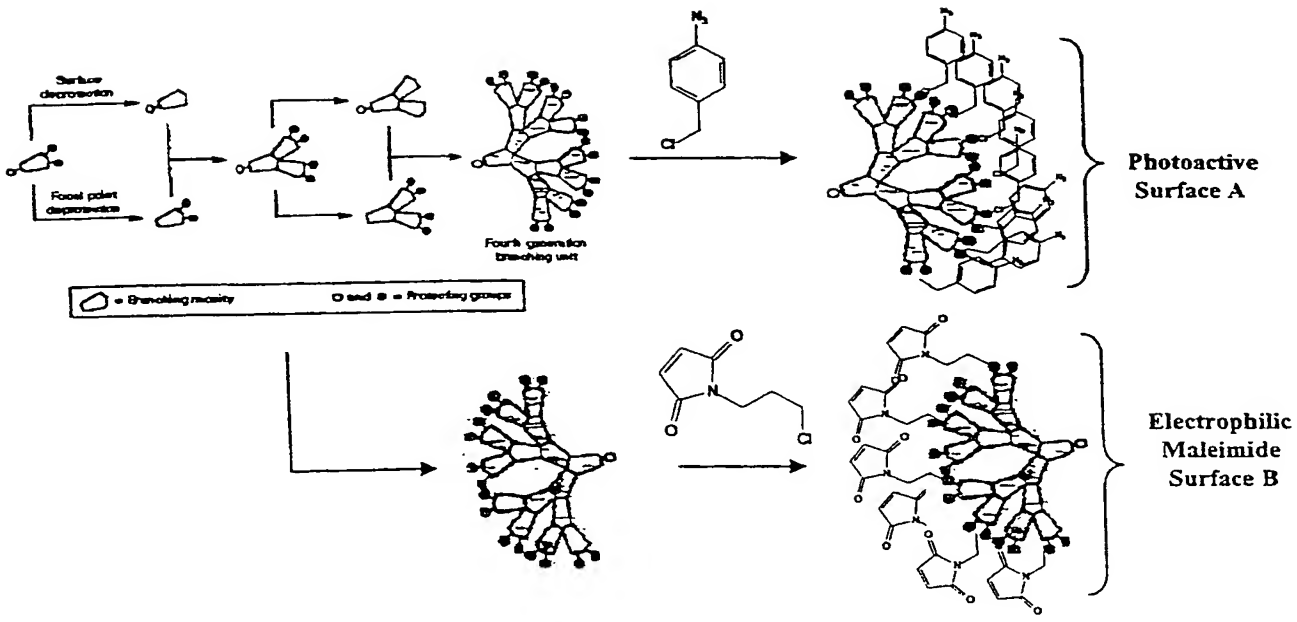


Figure 14

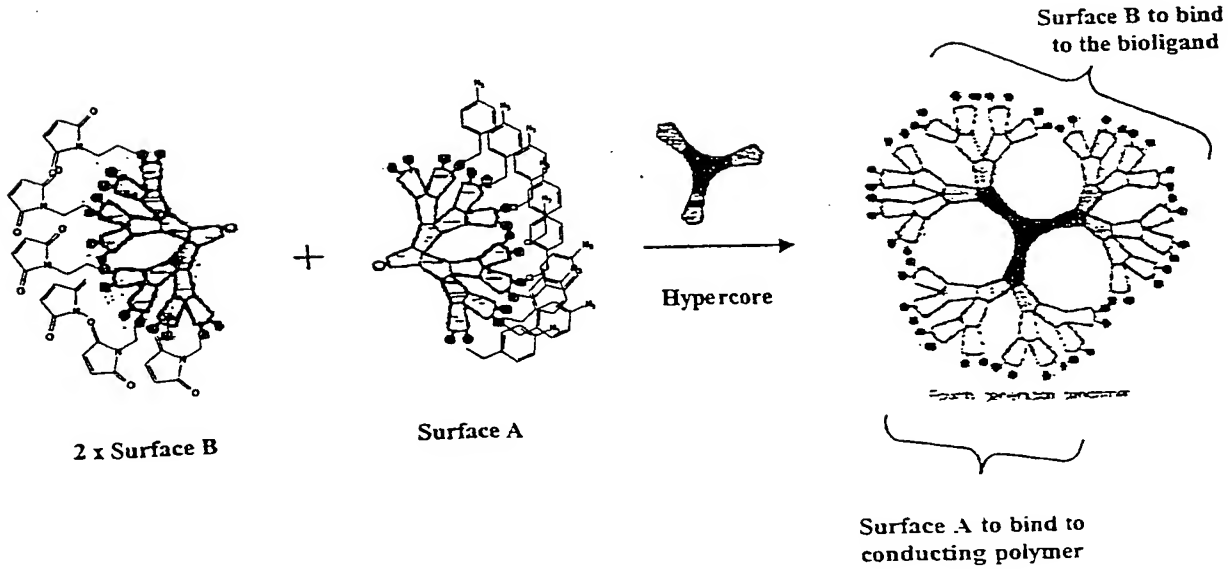


Figure 15

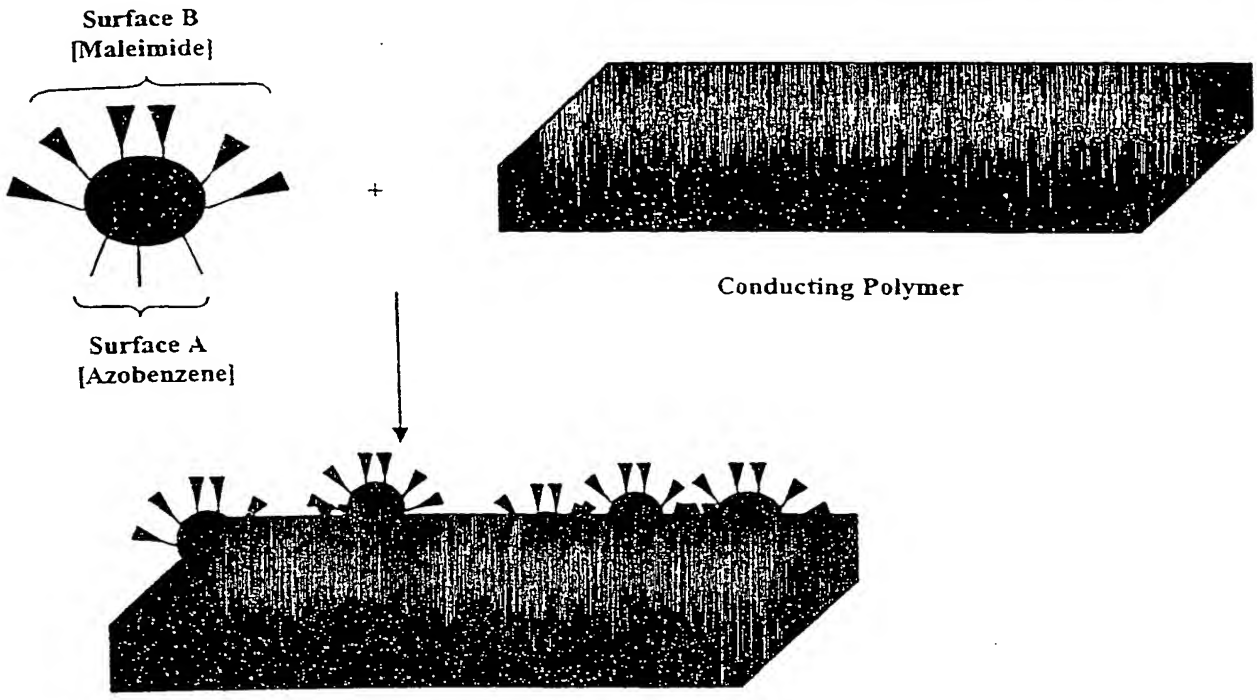
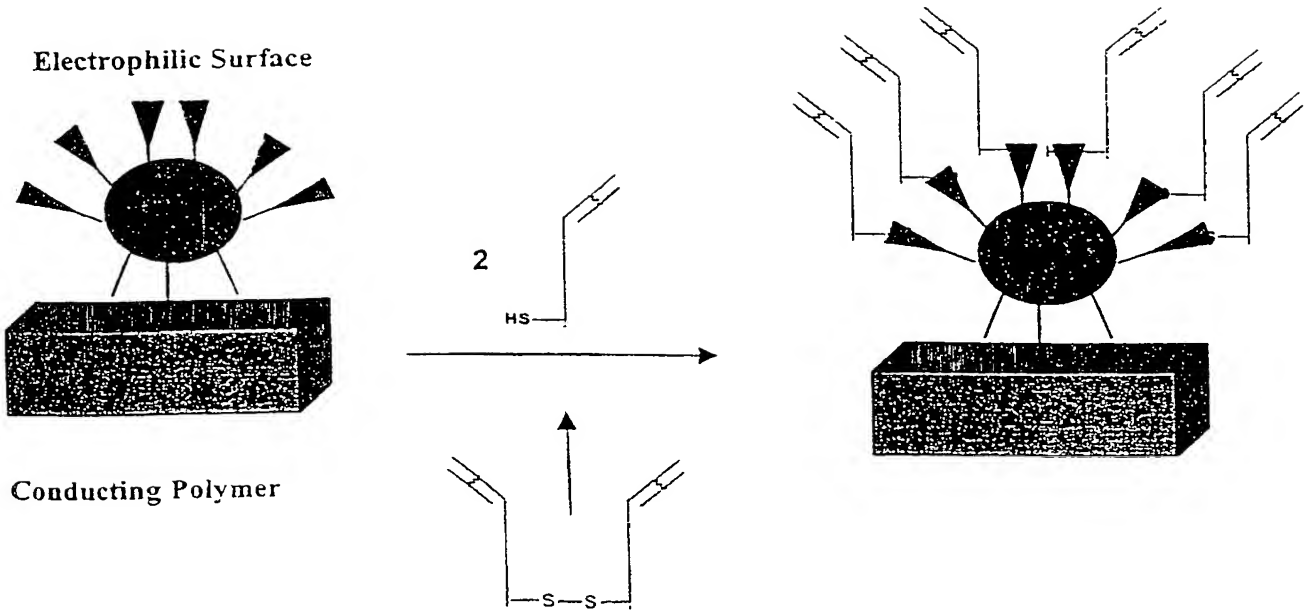


Figure 16



Antibodies Specific for Food Poisoning Agents

Figure 17

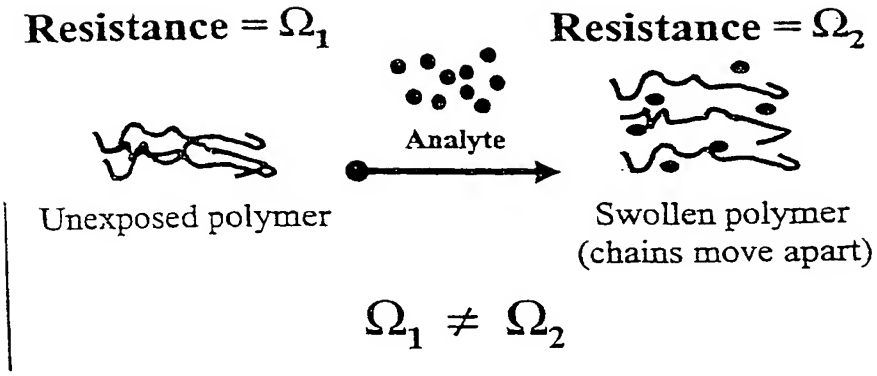


Figure 18

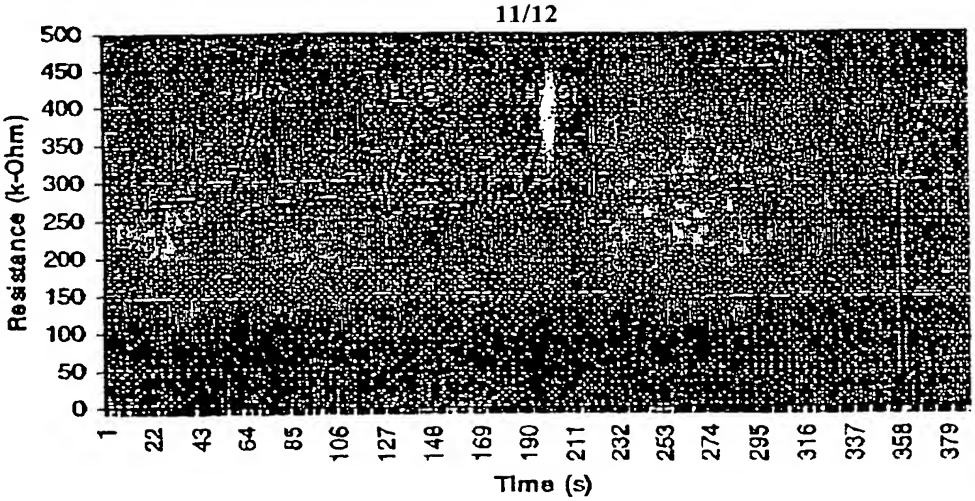


Figure 19A

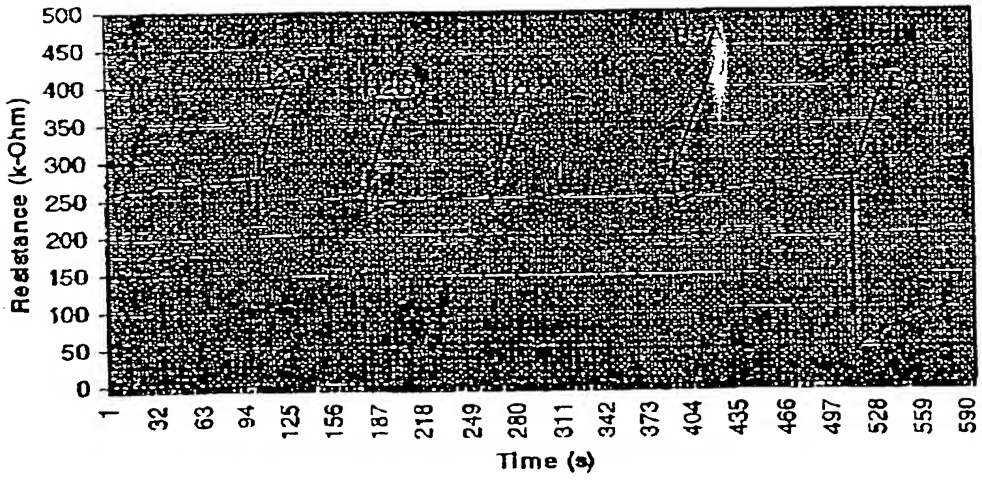


Figure 19B

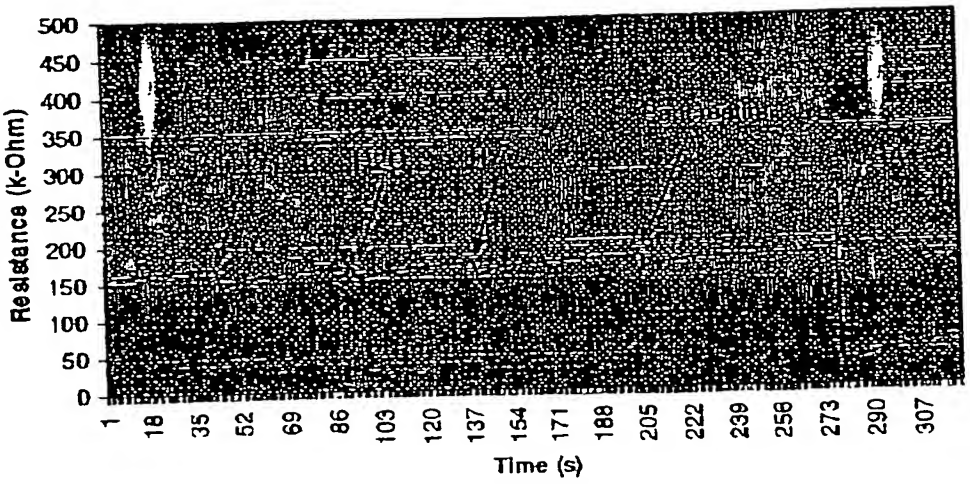


Figure 19C

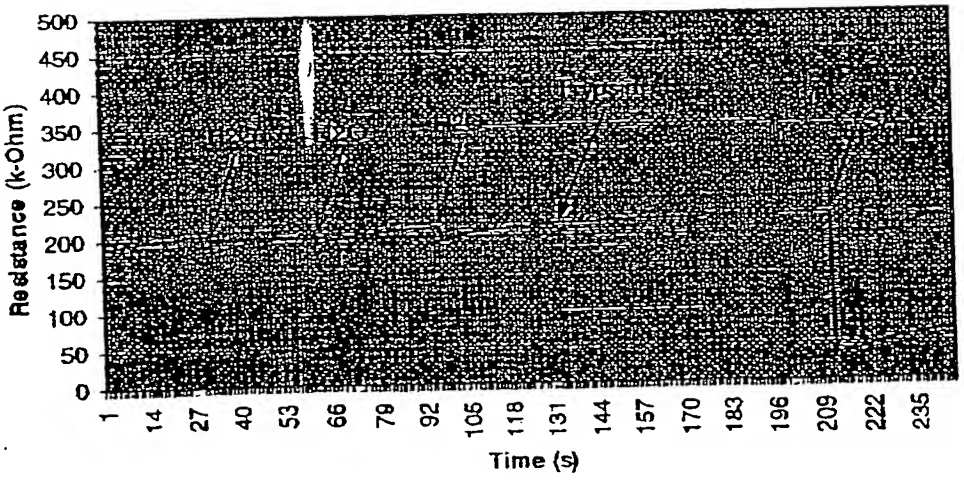


Figure 19D

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(71) Applicant: **TRITON SYSTEMS, INC.** [US/US]; 200 Turnpike Road, Chelmsford, MA 01824 (US).

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(74) Agents: **BUCHANAN, Robert, L.** et al.; Dike, Bronstein, Roberts & Cushman - IP Practice Group, Edwards & Angell, LLP, P.O. Box 9169, Boston, MA 02209 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(57) Abstract: Disclosed is an implementation for detecting at least one analyte in a sample. In one embodiment, the implementation includes a sensor array featuring one or a plurality of test sensors. Typically, eachof the test sensors includes a set of electrodes configured with a insulating surface to form a chamber. The implementation further includes a semi-conductive film positioned at least in the chamber; and a polyfunctional linker comprising a first end attached to a receptor and a second end. Also provided are methods for making and using the implementation.

INTERNATIONAL SEARCH REPORT
Form PCT/ISA/210 (second sheet) (July 1998)
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International application No.
PCT/US01/51917

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/00, 1/68, 1/34, 1/02; G01N 33/53; C12M 1/34
US CL :435/4, 6, 7.1, 18, 29, 287.1, 287.2, 811
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 6, 7.1, 18, 29, 287.1, 287.2, 811

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,444,892 A (MALMROS) 24 April 1984, see entire document.	1-79
Y	US 5,766,934 A (GUISEPPI-ELIE) 16 June 1998, see entire document, especially column 6, lines 4-10; column 23, lines 44-47 and column 25, lines 9-12.	1-79
Y	US 5,352,574 A (GUISEPPI-ELIE) 04 October 1994, see entire document, especially column 9, lines 60-65.	1-79
Y	US 4,716,122 A (SCHEEFERS) 29 December 1987, see entire document, especially column 2, lines 49-68.	13-30, 73
Y	US 5,788,989 A (JANSEN et al) 04 August 1998, see entire document.	31-37, 73

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 FEBRUARY 2002	Date of mailing of the international search report 09 APR 2002
Facsimile No. (703) 305-3230	Authorized officer AND Telephone No. DAVID M. NAFF (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
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B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

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search terms: semi-conductive, sensor, polymer, polyfunctional, electrode, analyte, chamber, bifunctional, receptor, photoactivatable, linker, dendrimer

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(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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(54) Title: SENSORS COMPRISING A SEMI-CONDUCTIVE POLYMER

(57) Abstract: Disclosed is an implementation for detecting at least one analyte in a sample. In one embodiment, the implementation includes a sensor array featuring one or a plurality of test sensors. Typically, eachof the test sensors includes a set of electrodes configured with a insulating surface to form a chamber. The implementation further includes a semi-conductive film positioned at least in the chamber; and a polyfunctional linker comprising a first end attached to a receptor and a second end. Also provided are methods for making and using the implementation.

SENSORS COMPRISING A SEMI-CONDUCTIVE POLYMER

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims benefit to U.S provisional application serial no. 60/240,152 as filed on October 14, 2000. The disclosure of the 60/240,152 provisional application is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention generally relates to implementations for detecting an analyte in a sample. In one aspect, the invention features sensors for detecting a wide spectrum of bioactive analytes including pathogenic cells and particles. Also provided are methods for making and using the implementations. The invention has many important uses including providing biosensors for the rapid detection of amino acid sequences as well as bacterial and viral pathogens and DNA.

BACKGROUND

There has been interest in developing sensors to detect analytes. Analytes of particular interest (sometimes called ligands) have included bioactive molecules, cells, and non-cellular bioactive particles such as viruses. Sensor detection of small molecules such as drugs and environmental contaminants have also attracted much interest. See generally Rogers, K.R. in *Mol. Biotech.*, 14: 109-129; (2000); Laval, J.M in *Analyst*, 125: 29-33 (2000); and Freitag, R. in *Curr. Opin. Biotech.* 4: 71-79 (1993).

One approach for detecting analytes has been to use a receptor (sometimes called a binding partner or ligand) to bind the analyte. A goal is to form a binding complex between the receptor and the analyte. Formation of the complex can be specific in the sense that the receptor only binds the analyte, or it can be less specific, meaning that the receptor can bind other molecules in addition to the analyte. In many but not all instances, the receptor is selected to specifically bind the analyte and form a specific binding complex. Sensor detection of the binding complex is taken to be indicative of presence of the analyte in a sample of interest. Absence of the

binding is taken to mean that the analyte is absent in the sample or present in an amount that is below the sensitivity or selectivity of the sensor.

There have been efforts to develop specific sensors for detecting analytes.

For example, some sensors have been designed to output a detectable signal, usually electronic, when a desired analyte is present in the sample. Such a sensor typically includes electronic components that measure voltage (potentiometric), current (amperometric), light, sound, temperature or mass (piezoelectric) differences produced by formation of the binding complex. See e.g., Lowe, C. R., *Biosensors* 1: 3-16(1985) and Wohltjen, J., *Analytical Chemistry* 56:87-103 (1984).

More specific sensors have been referred to as biosensors, bioaffinity sensors and the like. In particular, there have been reports of enzyme-based and metabolic biosensors. Both types are disclosed as relying on enzymatic or metabolic processes to detect a reaction product. That product often arises through catalytic contact between the enzyme and its substrate.

An example of an enzyme-based biosensor is one that uses glucose oxidase or urease based electrodes. Such enzyme electrodes are known and many are commercially available. See e.g., Vadgana, P., *Journal of Medical Engineering Technology*, 5: 293-298 (1981); Solsky, R.6., *CRC Critical Review of Analytical Chemistry*, 14:1-52 (1983).

See also Taylor, R.F., *The World Biotech Report* 1986, Vol. 2, pp.7-18 (1986) for a discussion of biosensors that are reported to use an immobilized receptor (e.g., an antibody). Analyte binding to the receptor is thought to produce a detectable change in the shape or conformation of the receptor. This change is disclosed as manifesting an output signal that is indicative of the presence of the analyte in the sample. As reported, the change can be optical (interference, refractive index, fluorescence, etc), mechanical (mass or density) or temperature dependent.

See also U.S. Pat. Nos. 5,352,574 and 5,001,048 (disclosing other immobilized receptors for detecting an analyte).

Particular attention has focussed on sensors with antibody-based receptor molecules. See e.g, Aizawa, M., et al., *Journal of Membrane Science* 2:125-132 (1977). In this instance, binding between the receptor and antigen is disclosed as producing a registerable electrical change.

Other biosensors are known. For example, there have been reports of optical biosensors based on antibody-antigen binding. As disclosed, the biosensor works by communicating a change in receptor conformation, the receptor environment, or both. The reported signal changes can be detected and amplified using conventional transducer technology. See Place, J. F., et al., *Biosensors* 1:321-353 (1985).

Others have described efforts to immobilize receptors and particularly enzymes on surfaces such as glass (U.S. Pat. No. 4,357,142), polymer surfaces (U.S. Pat. Nos. 4,352,884; 4,371,612; 5,897,955), and protein films (U.S. Pat. No. 5,001,048).

More recently, biosensors that employ a semi-electroconductive polymer to transduce a signal have been disclosed. For example, see Uchida, I.J. *Electroanal. Chem. Interfacial Electrochem.* 300: 111 (1991); and U.S. Pat. Nos. 5,766,934; 5,312,762 (and references cited therein).

There have been problems associated with many of the prior sensors.

For example, a significant drawback has been the inability of many prior sensors to detect analyte binding in less than a few minutes. This problem negatively impacts sensor performance in many ways. Specifically, such sensors may not always be able to analyze flow stream samples very well. In addition, real-time output to a user may be compromised especially in medical settings in which rapid analyte detection is needed for optimal patient care.

Additionally, many of the prior sensors have been plagued by less- than-adequate signal-to-noise ratios. This has impeded good sensor performance especially when samples having low (trace) amounts of analyte are measured. Samples that have signal interfering substances have been difficult to analyze using such sensors.

Many of the prior sensors also have problems outputting a useful signal to a sensor end user. More specifically, there have been difficulties achieving reliable and effective signal transduction from the receptor-analyte binding complex to an attached conductive polymer. This problem has caused many shortcomings including loss of sensor sensitivity and/or selectivity.

There have been attempts to resolve some of these problems.

For example, there have been reports of sensors in which the receptor (sometimes termed the binding agent) is soaked into or co-polymerized with a semi-conductive polymer. See U.S. Pat. Nos. 4,444,892; and 5,192,507. However, as reported, the relationship between the receptor and the polymer combined in this manner is not believed to be optimal. More particularly, such sensors are not believed to associate the receptor and polymer in a way that provides for effective signal transduction. Implementation sensitivity and user satisfaction are often compromised as a result. Significantly, it is believed that use of such sensors can often mask analyte binding sites, thereby decreasing sensitivity and/or selectivity of the sensor in many instances.

There have been other difficulties associated with many of the prior sensors.

For example, it has been difficult to establish a suitable receptor density in many instances. This drawback has impeded good sensor performance.

For example, sensors with an inadequate receptor density may not always be sensitive or selective enough for particular analyte samples. Such sensors may not always be able to detect analytes reproducibly or with an acceptable cost/benefit ratio.

In addition, many of the prior sensors may not always be able to detect trace analytes or analytes having short lifetimes. In many medical settings, this problem can be especially grave resulting in misdiagnoses and jeopardized patient care.

Sensors having unsuitable receptor densities may especially suffer from low sensitivity and reliability particularly when good analyte binding relies on interaction with more than one receptor.

Significantly, many of the prior sensors are not compatible with miniaturization strategies that are often needed for optimal use. Sensor miniaturization is especially needed for applications involving many diagnostic, commercial, robotic, remediation, research and medical applications.

It would be desirable to have sensors featuring especially good signal transduction between the receptor and the polymer. It would be particularly desirable to have sensors with good contact relationship between the receptor and the polymer to facilitate efficient signal transduction. Further, it would be desirable to have sensors and especially biosensors that can be miniaturized and can output signal to an end-user in less than a few minutes.

SUMMARY OF THE INVENTION

The invention generally relates to an implementation for detecting at least one analyte in a sample as well as methods for making and using the implementation. More specifically, the invention relates to an implementation that provides sensitive and rapid detection of a wide range of analytes such as bioactive molecules, particles and cells; and small molecules. The invention has a variety of important uses including the detection of amino acid sequences as well as cell and viral pathogens such as those implicated in food poisoning, tuberculosis, and pediatric infections.

Particular implementations of this invention feature a sensor array that includes one or a plurality of sensors adapted to detect the analyte (or class of analytes). Each sensor in the array typically includes a set (often less than about ten and usually less than about five or six) of electrodes configured with a suitable

insulating surface to form a chamber. Preferably, a semi-conductive film is positioned in at least the chamber (including, for example, other portions of the sensor or sensor array) sufficient to place the film in electrical contact with the electrodes. As discussed below, it is an object of the invention to place the semi-conductive film in effective contact relationship with at least one analyte binding receptor. Generally, that effective contact relationship is provided by positioning the receptor and the semi-conductive film with a specific linking group as described below.

In embodiments in which the implementation includes more than one analyte binding receptor, each receptor can bind the same or different analyte including a class of analytes. Further, each receptor can be the same as or different from another receptor in the sensor array. Choice of a particular receptor or group of receptors will be generally guided by intended use of the implementation including the analyte (or class of analytes) for which detection is desired.

A preferred semi-conductive film according to the invention is sensitive to analyte binding to the receptor. Preferably, it registers that binding as a change in at least one electrical property of the film. Typically, the change is outputted to an end-user as a detectable signal. That signal is taken to be indicative of the presence of the analyte in the sample. Of course, in cases in which there is no detectable signal, that will often mean that the analyte is not present in the sample.

More specifically, we have found that by providing the good contact relationship between the receptor and the semi-conductive film it is possible to enhance implementation performance in many ways.

For example, the ability of the invention to provide the good contact relationship has been found to improve signal transduction i.e., communication between the receptor and the semi-conductive film. This positively impacts performance of the implementation e.g., by helping to boost sensitivity and selectivity for the analyte. Samples with trace or labile analytes can now be detected efficiently and with an acceptable cost/benefit ratio. Moreover, the good contact relationship helps to minimize implementation response times to less than a few minutes. Such

improved response times facilitates signal output to an end user with less delay and often in real time. This feature improves implementation performance as when the sample is presented as a flow stream or when output results are urgently needed.

The invention generally achieves the effective contact relationship by employing a particular polyfunctional linker between the receptor and the semi-conductive film. By the term "polyfunctional" is meant that the linker can bind more than one binding partner, which partner includes the receptors and semi-conductive films described herein.

A particular polyfunctional linker is configured to join (either covalently or non-covalently) at least one receptor to the semi-conductive film. As will be appreciated, the precise number of receptors joined is usually determined by intended use of the implementation. More preferred polyfunctional linkers suitably space the receptor from the film, thereby helping to optimize binding contact between receptor and analyte.

More specific polyfunctional linkers according to the invention include at least two reactive groups which groups are usually pre-determined to bind (either covalently or non-covalently) the receptor and/or semi-conductive film to the linker. Such preferred polyfunctional linkers often include a "rod-like" component having minimal freedom of movement. An advantageous feature of the complex formed between the receptor, the polyfunctional linker and the semi-conductive film is that the complex serves as an effective conduit for transducing signal from the receptor to the semi-conductive film. That signal can, for example, be electrical, mechanical or a combination thereof. A preferred semi-conductive film is one that can effeciently receive signal from the polyfunctional linker and propagate same (usually at least in part as an electrical signal) towards an appropriate detector and ultimately to the end-user of the implementation.

The good contact relationship provided by the polyfunctional linkers of this invention provide other advantages.

For example, it has been found that when at least the polyfunctional linker is present at a particular density on the film, it is possible to produce an implementation with very favorable performance characteristics. Without wishing to be bound by any theory, it is believed that when the polyfunctional linkers of the invention are suitably close together on the film and with at least a part of the linker in signalling contact with the film, it is possible to transduce signal from the receptor not only vertically (ie. from the receptor to the semi-conductive film) but also horizontally (ie., to another receptor, polyfunctional linker, or both; followed by signal transduction to another part of the film). This feature of the invention can result in a horizontal "domino effect" that helps increase sensitivity to the analyte. Such a domino effect can, in some embodiments, facilitate signal amplification, thereby helping to assist with the detection of many analytes such as those that have short lifetimes or are present in trace quantities.

Sometimes, a suitably dense configuration of polyfunctional linkers will be referred to herein as an aggregate or network of aggregates of polyfunctional linkers.

More specific polyfunctional linkers of the invention include reactive groups usually positioned at at least one end of the linker. In embodiments in which the polyfunctional linker has at least two of such reactive groups, they can be the same but in most cases they will be different from each other. Other formats are within the scope of this invention as when at least one reactive group is attached to the linker between the ends of the linker or attached to the linker by one or more chemical moieties. For most applications however, a first reactive group will be positioned at a first end of the polyfunctional linker, while at the other (second) end, a second reactive group will be placed. Typically, but not exclusively, the first and second reactive groups will be chemically different from one another.

A preferred second reactive group is adapted to provide effective bonding between the polyfunctional linker and the semi-conductive film. Typically, such a preferred group will be chemically reactive. Preferably, it will also be controllably activatable. By the term "controllably activatable", it is meant that one can readily

control the amount and/or extent of reaction between the polyfunctional linker and the semi-conductive film by applying a controllable reaction stimulus.

For example, in invention embodiments in which very dense receptor aggregates (including networks of aggregates) are desired, controllable activation of at least the second chemically reactive group will be highly beneficial. In this instance, such activation allows the user to first generate aggregates or networks having high densities. Such controllable activation also allows the user the freedom to first make multiple networks having two or more different receptors. This feature of the invention provides significant advantages including allowing the manufacture and use of implementations with customized receptor networks having a pre-determined density.

More preferred second chemically reactive groups are generally sensitive to a controllable stimulus that can be initiated e.g., by the implementation user. In this invention embodiment, it is often but not exclusively preferred that the first end of the polyfunctional linker not be as sensitive to that stimulus. Preferably, the first end is essentially insensitive to the stimulus. Particular stimuli of interest are light and temperature. In cases in which the second chemically reactive group is sensitive to light, it is preferred that the group be photoactivatable.

In embodiments in which the second end of the polyfunctional linker is photoactivatable, an especially good contact relationship can be achieved between the receptor and the semi-conductive film. In particular, it has been found that photoactivation of such polyfunctional linkers produces a plurality of covalent bonds that positively impact effective signal transduction. More particularly, such photoactivatable polyfunctional linkers have been found not only to anchor the receptor to the film but also to help form an efficient signal conduit between the receptor and the film.

As discussed, preferred polyfunctional linkers of the invention feature a first chemically reactive group, which group is usually, but not exclusively, positioned on the first linker end and which is often, but not exclusively, insensitive to the stimulus

for activating the second chemically reactive group. The first chemically reactive group is generally flexible in the sense that it can support a range of attachment strategies between the polyfunctional linker and the receptor. Choice of one strategy in lieu of another will be guided by recognized parameters including the receptor and analyte to be detected, the level of sensitivity and/or selectivity desired, and the type or quantity of sample to be analyzed. More preferred first chemically reactive groups will support covalent bonding between the polyfunctional linker and the receptor. Additionally preferred groups will be capable of bonding the receptor to the polyfunctional linker by non-covalent means, specifically by at least one of hydrogen bonds, ionic bonds (e.g, salt bridges), hydrophobic interactions and Van der Waals forces. In one invention embodiment, the first chemically reactive group includes at least one electrophilic group capable of covalently or non-covalently bonding the receptor to the polyfunctional linker.

More specific polyfunctional linkers in accord with the invention are suitably amphiphilic (i.e., have an affinity for water and lipid in the same molecule). In this embodiment, such polyfunctional linkers are well suited for associating with aqueous and non-aqueous environments. For example, such a linker can include a polar head group (typically, but not exclusively, as the first chemically reactive group), an adjustable bridge, and a hydrophobic group or tail (typically, but not exclusively, as the second chemically reactive group). In one embodiment, the polar head group is covalently bound to the hydrophobic tail by the adjustable bridge. In this example of the invention, the polyfunctional linker provides an especially good contact relationship between the receptor (usually in a polar environment) and the semi-conductive film (often in a much less polar environment, usually hydrophobic). Significantly, the bridge provides an effective and adjustable spacing between the receptor and film environments. By the word "adjustable", it is meant that the bridge can be designed as needed to suitably space the receptor from the semi-conductive film. Significantly, the length of the adjustable bridge can be customized to suit an intended use or spectrum of different uses.

Accordingly, and in one aspect, the invention features an implementation for detecting at least one analyte in a sample (including a class of analytes as well).

Preferably, the implementation comprises a sensor array which array includes one or a plurality of operably linked test sensors. Also preferably, each test sensor includes at least one and typically all of the following components:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iii) a polyfunctional linker comprising a first end attached to the receptor (covalently or non-covalently) and a second end attached to the semi-conductive film (covalently or non-covalently).

In a particular embodiment of the foregoing implementation, the semi-conductive film includes at least one polymer material that provides the attachment to the second end of the polyfunctional linker. Preferably, the second end of the linker is pre-selected to provide good contact relationship between the receptor and the semi-conductive film. Methods for selecting such polyfunctional linkers, as well other implementation components such as an appropriate semi-conductive film are described below. In general, such methods use what is referred to below as a standard streptavidin biosensor.

Especially good contact between the receptor and the semi-conductive film is typically manifested between the second end of the polyfunctional linker and the polymer material. In most cases, the second end is covalently bonded to the backbone of the polymer material. However, in some invention embodiments effective contact can include covalent or non-covalent bonding to at least one chemical group (linking moiety) attached to the polymer backbone. As discussed below, such good contact can also be provided when the second end is intercalated within the polymer material or when it is non-covalently associated with the polymer material. As discussed, especially preferred polyfunctional linkers also effectively space the receptor from the film while providing an effective conduit for signal transduction to the film.

Also preferably, the electrode set described above will include electrodes comprising or consisting of a substantially non-corrosive metal or metal alloy. A preferred metal is gold or other suitable metal.

Choice of a particular semi-conductive film to use in accord with the invention will be guided by recognized parameters including intended use. A preferred film is generally conducive to registering analyte binding as a change in at least one electrical property of the film including, but not limited to, resistance, impedance, capacitance, and conductivity. That change is taken by the end-user to be indicative of the presence of the analyte in the sample.

It is an object of this invention to provide flexible implementations that can be customized to suit a desired application or group of applications.

For example, in one embodiment, the implementation includes a detector for receiving and detecting signal passing through the semi-conductive film. That detector is operably linked to at least one of the sensors in the array. Preferably, the detector is adapted to output any change in the electrical property to a user of the implementation. The term "operably linked" is meant to indicate that a given implementation component has a functional relationship with at least one other such component. That functional relationship can be served by integrating the component into the implementation. Alternatively, the component may be in a stand-alone configuration with the implementation.

By way of example and not limitation, the implementation detector can be a "stand-alone" component of the implementation or it can be wholly or partially integrated into the implementation. In embodiments in which miniaturization is desired, integration of the detector and other implementation components will be important and sometimes essential for optimal use of the implementation.

A particular detector for use with the implementation is adapted to receive electrical or light signals outputted by the semi-conductive film. Such detectors are standard in the field and include an ohm meter, capacitance meter and the like.

More specific implementations include a plurality of interdigitated electrodes. In an example, the interdigitated electrodes are each connected to the detector. Preferably, the detector is operably linked to a power supply that can supply the implementation with a power source.

The semi-conductive film can be placed in the implemenetation in one or a variety of ways to help achieve good analyte detection. In one embodiment, the semi-conductive film comprises at least one layer of polymer material, typically between from about 2 to about 10 of such layers. Methods for making such layers including a preferred "layer-by-layer" approach are described below in more detail.

The invention is compatible with use of a variety of different semi-conductive films. Choice of one of the films will be guided by intended use. Also envisioned are blends of individual semi-conductive films (polymer blends). For example, a preferred semi-conductive film will typically exhibit reactivity sufficient to participate in chemical bonding with the polyfunctional linker, usually but not exclusively at the second end. In many embodiments, the polymer material of the film will bond to the polyfunctional linker end covalently although in some examples of the invention non-covalent binding will be preferred.

In more specific embodiments of the invention, a suitable semi-conductive film is further selected to manifest an increase or decrease in electrical conductivity between the electrodes in the presence of the analyte. Alternatively, the film can be selected to manifest an increase or decrease in one or more of capacitance or impedance, in the presence of the analyte.

As mentioned previously, methods for testing performance of a variety of implement components e.g., the semi-conductive film, polyfunctional linker, and receptor, are provided below. A preferred method involves use of a streptavidin-alkaline phosphatase sensor described below in Example 1. As discussed below, one or more components of the streptavidin-alkaline sensor can be replaced with a desired "test" component to evaluate performance in the sensor. Accordingly, the

streptavidin-alkaline sensor can be used to test the functionality of a wide range of semi-conductive films, polyfunctional linkers, and other implementation components to identify those components having desired performance characteristics.

The implementation of the present invention provides other significant advantages.

For example, in preferred embodiments, the implementation provides means for rapidly detecting and identifying analytes often in less than about 2 minutes down to about real time. Generally, the implementation can be assembled at low or moderate cost while providing good sensitivity and selectivity for one or a class of analytes. Significantly, the invention provides essentially reagentless-detection. That is, optimal practice of the invention usually requires no additional materials, such as analyte tags or probes. Also significantly, preferred implementations are easy to use and require low power. In particular embodiments, the implementation is reusable and can be provided with a disposable low-cost sensing element. In embodiments in which the implementation is fully or partially miniaturized, the implementation can serve as a portable, handheld device.

In another aspect, the invention provides methods for detecting an analyte in a sample. In one embodiment, the method includes contacting the sample with an implementation for detecting the analyte or class of analytes. A preferred implementation for practicing the methods includes at least one and preferably all of the following components:

a. a sensor array comprising one or a plurality of test sensors in which each test sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iii) a polyfunctional linker comprising a first end attached to a receptor and a second end.

In a preferred example of the method, the semi-conductive film includes at least one polymer material attached to the second end of the polyfunctional linker. Typically, the film is selected to be conducive to registering analyte binding as a change in at least one electrical property of the film. Preferably, that change is indicative of the presence of the analyte in the sample.

The implementations of this invention can be made by one or a combination of different approaches. For example, in one approach, the implementation can be made by at least one and preferably all of the following steps: layering the semi-conductor film within the chamber, contacting a polyfunctional linker with the film under conditions sufficient to bind the linker to the film surface or within the film; and contacting the receptor to the polyfunctional linker under conditions sufficient to bind the receptor to the polyfunctional linker to make the implementation.

The implementation can be made by other means including at least one and preferably all of the following steps: layering the semi-conductor film within the chamber, contacting a polyfunctional linker with a receptor under conditions sufficient to form a linker-receptor binding complex; and contacting the linker-receptor binding complex to the film under conditions that bind the polyfunctional linker to the film surface or within the film to make the implementation.

In the foregoing methods for making the implementation, conditions sufficient for binding the polyfunctional linker to the semi-conductive film and/or for binding the receptor to the polyfunctional linker generally involve providing reaction conditions conducive to making covalent or non-covalent bonds with the polyfunctional linker. As illustrations, the polyfunctional linker can be covalently bound to the semi-conductive film at one end and the receptor at the other end thereof. However in another embodiment, one end of the polyfunctional linker can be non-covalently bound to the semi-conductive film or be intercalated therein while at the other linker end, the receptor can be covalently or non-covalently bound to the linker. For many invention applications, the receptor will be covalently bound to the polyfunctional linker which linker will preferably be covalently bound to the semi-conductive film.

In more particular embodiments of the foregoing methods, the polyfunctional linker is configured as a thin film prior to or during binding (covalent, non-covalent, or by intercalation) to the semi-conductor film.

In another more particular embodiment, the methods further include making the thin film using a Langmuir-Blodgett device and transferring the thin film from the device to the insulating surface of the implementation. This feature of the invention provides important advantages including providing exceptionally thin and uniform films for use in the implementation. Such thin films have a variety of desirable performance characteristics including the capacity to lend good resistance, capacitance, and/or impedance features to the implementation.

In a particular embodiment of the foregoing methods, the step of layering the semi-conductive film in the chamber can be repeated as needed, usually at least once, to form a multi-layer film having a thickness of from between about 10 Angstroms to about 1000 Angstroms, preferably about 50 Angstroms to about 100 Angstroms. Preferred practice of this method is sometimes referred to herein as a "layer-by-layer" approach.

Other aspects and advantages of the invention are described *infra*.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing showing biospecific binding of Streptavidin-Alkaline Phosphatase to a ligand-functionalized conductive polymer-based sensor. Binding of the biomolecule is thought to "twist" the underlying polymer chains and change the electrical resistance of the conductive film.

Figure 2 is a drawing showing exposure of a particular sensor of the invention to a target for which it was intended. A detectable drop in the electrical resistance of the sensor is observed.

Figure 3A-B are drawings showing covalent attachment of densely-packed bioconjugate array and mechanism of signal generation on binding analyte. (3A) Bioconjugates are photochemically attached to conducting polymer substrate. (3B) Conducting polymer is perturbed when analyte binds to ligands (not drawn to scale). Polymer conductivity changes.

Figure 4 is a drawing showing synthesis of a preferred bifunctional amphiphilic bioconjugate (linker).

Figure 5 is a drawing showing manufacture of a Langmuir-Blodgett film. An amphiphile solution is spread out on the water surface (in trough). A moveable barrier compresses the amphiphile monolayer to the "condensed liquid" state.

Figure 6 is a drawing showing a preferred (Schaefer) method for transferring a Langmuir-Blodgett fim to a conducting polymer surface.

Figure 7 is a drawing showing construction of one sensor embodiment. In the drawing, step 2 refers to making an antibody component (see text).

Figures 8A-B are schematic drawings showing (8A) mushroom shaped aggregate of rationally designed linkers and (8B) linkers intercalated into a conductive polymer film for signal amplification.

Figure 9 is a schematic drawing illustrating synthesis of a modified rod-coil structure according to the invention

Figure 10 is a schematic drawing showing formation of amphiphilic nanostructures and intercalation into a conducting polymer.

Figure 11 is a schematic drawing showing covalent bonding of antibody fragments to an intercalated rod-coil nanostructure according to the invention

Figures 12A-B are drawings generally showing use of dendrimers according to the invention. (12 A) Ligand-functionalized dendrimers attach to substrate using multiple photoreactive arms. (12 B) Conducting polymer is perturbed when analyte binds to ligand (not drawn to scale). Polymer conductivity changes.

Figure 13 is an illustration showing a general scheme for dendrimer synthesis

Figure 14 is a drawing showing initial mixed convergent synthesis of surface A (photoactive) and surface B (electrophilic).

Figure 15 is a drawing illustrating synthesis of a bifunctional dendrimer

Figure 16 is a drawing showing conjugation of bifunctional dendrimer to a conducting polymer.

Figure 17 is a drawing showing a complete bioligand linked to a surface of a conducting polymer.

Figure 18 is a graph illustrating swelling of a conductive polymer film by an ingressing species causes a change in the electrical conductivity of the film.

Figures 19A-19D are graphs showing a drop in biosensor resistance in the presence of ligand but not the following interferents: (19A) lysozyme, (19B) bovine serum albumin, (19C) pancreatin, and (19D) trypsin.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention relates to implementations for detecting a substance and more specifically to receptor-based sensors for the identification and quantitation of an analyte (or class of analytes) in a sample of interest. Also provided are methods of making and using the sensors. The sensors of this invention, sometimes called biosensors or bioaffinity sensors, typically include at least one receptor for binding the analyte, at least one polyfunctional linker for binding the receptor, and a semi-conductive film comprising at least one polymer material bound.

As discussed above, it is an object of this invention to provide sensors with good contact relationship between the semi-conductive polymer, polyfunctional linker and receptor. Such a sensor provides many advantages including providing for efficient signal transduction between the receptor and the semi-conductive film and good signal output to the end-user.

More preferred sensors of this invention can be used to detect, identify and quantify an analyte of interest in a wide range of samples e.g, liquid, high-water gel, air, mist, vapor, gas such as air, or emulsion sample. In embodiments in which the sample is gaseous (or partially gaseous), known techniques for transfer of an air sample to a liquid stream can be used to provide a liquid sample for use with the sensor.

By the phrase "class of analytes" is meant ligands having similar chemical or physical structures such that one receptor can bind the ligands either specifically or non-specifically. Such "class" binding is a basis for drug activity and toxicity of many substances. See e.g., U.S. Pat. No. 5,001,048 (disclosing analyte classes e.g., drugs, organophosphorus compounds, viruses, hormones, toxins, and environmental chemicals and pollutants that bind the acetylcholine receptor).

In one sensor of the invention, a pre-selected receptor is bound (covalently or non-covalently) to a suitable polyfunctional linker including those specific bifunctional linkers and dendrimeric linkers disclosed herein. That polyfunctional linker is subsequently bound (covalently or non-covalently) to an appropriate semi-conductive film such as those described below. In embodiments in which the polyfunctional linker is bifunctional, such a linker typically includes what is referred to as a "rod-like" component which component preferably has a height of from between about 0.1nm to about 100nm, preferably about 0.5 nm to about 50 nm. In this example of the invention, the bifunctional linker has a preferred molecular weight of between from about 0.5 kDa to about 100 kDa as determined by gel permeation chromatography or other suitable assay with between about 1 kDa to about 20kDa being preferred for many applications.

As discussed, the sensors of this invention are compatible with a wide variety of suitable bifunctional linkers.

For example, one such linker has one end (terminus) with a photoactivatable group such as an azide group. Such an end preferably can undergo nucleophilic/electrophilic conjugation to or with a desired analyte (ligand molecule). Another preferred linker is between from about 2.0 to about 500.0 nm in length. An additionally preferred linker has an overall shape of a distorted cylinder with a preferred diameter of from about 1nm to about 5 nm. More preferably, the overall length of the linker is less than 50 nm and the van der Waals volume is preferably between from about 60 to about 150 nm^3 as determined by light scattering. Most preferably, the overall length of the linkers would be less than about 50 nm and the linkers would be rigid with a radius of gyration from about 2 to about 10 nm (as determined by light scattering) and the ligands would form a self-assembled monolayer

Additionally preferred linkers of the invention are organic or at least semi-organic and include a plurality of carbon-carbon, carbon-nitrogen or carbon-oxygen bonds. Typical of such linkers are those having a group at one terminal that is preferably, but not exclusively, photoactive such as an azido function, an azo function, a carbonyl group, and imine group, a thio group. Additionally preferred polyfuntional linkers have a group at one end (terminal) that preferably, but not exclusively, acts as an electrophile such as a succinimidyl, carbonyl, or a nucleophile such as an amine, hydroxyl, or a halogen.

More specific bifunctional linkers in accord with the present invention include, generally prior to reaction with the receptor and the semi-conductive film, an electrophilic group on the first end of the linker and a photoactivatable group on the second end of the linker. In one example of such a linker, the receptor is covalently attached to the electrophilic group of the bifunctional linker. Typically, a suitable polymeric material of the semi-conductive film is covalently bound to the photoactivatable group of the bifunctional linker. Typically, but not exclusively, the

electrophilic group of the bifunctional linker is polar and the photoactivatable group is hydrophobic.

It will be apparent that the invention is not limited to particular bifunctional or other linkers having chemically specified first and second ends. Accordingly, the linker can include, prior to reaction with the receptor and the polymeric material of the semi-conductive film, a photoactivatable group on the first end and an electrophilic group on the second end of the linker. Often, but not exclusively, the bifunctional linker is attached to the backbone (ie., main polymer chain) of the polymeric material. However in some invention embodiments it may be more desirable to covalently bind the linker to the backbone through a chemical linking group such as those with a joining hydroxyl, keto, carboxyl, ester, cyano or amino group. Choice of whether to include the linking group will be guided by recognized parameters such as intended use of the sensor and may include therefor other suitable chemical linking groups as needed. See e.g., U.S. Pat. No. 5,766,934 for disclosure about making specific polymer linking groups.

In one embodiment, the photoactivatable group of the bifunctional linker is an optionally substituted azobenzene group. In another invention embodiment, the electrophilic group is an optionally substituted maleimide group. Specifically preferred bifunctional linkers are amphiphilic and generally have water and lipid soluble portions.

By the phrase "optionally substituted" as used herein is meant substitution of hydrogen on a particular chemical group with another substituent with the proviso that such a substitution does not significantly impede the function for which the sensor was intended. Specifically, such substitution should not hinder binding between sensor components e.g., the polyfunctional linker and the receptor, the semi-conductive film and the polyfunctional linker, ect. Examples of such groups include substitution at available positions, typically 1 to 4 or 5 positions, by one or more suitable groups such as those disclosed herein. Suitable groups particularly include halogen such as fluoro, chloro, bromo and iodo; cyano; hydroxyl; nitro; azido; alkanoyl such as a C_{1-6} alkanoyl group such as acyl and the like; carboxamido; alkyl

groups including those groups having 1 to about 6 carbon atoms; alkenyl and alkynyl groups including groups having one or more unsaturated linkages and from 2 to about 6 carbon, or 2, 3, 4, 5 or 6 carbon atoms; alkoxy groups having those having one or more oxygen linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those moieties having one or more thioether linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; alkylsulfinyl groups including those moieties having one or more sulfinyl linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; alkylsulfonyl groups including those moieties having one or more sulfonyl linkages and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5, or 6 carbon atoms; and aminoalkyl groups such as groups having one or more N atoms and from 1 to about 6 carbon atoms, or 1, 2, 3, 4, 5 or 6 carbon atoms; carbocyclic aryl having 6 or more carbons, particularly phenyl.

More particular bifunctional linkers in accord with this invention can be represented by the following Formula I:

A-B-C

Formula I

in which A is defined as the an optionally substituted maleimide group, B is defined as a spacer having a length of from between about 0.1 Angstrom to about 100 Angstroms, preferably from about 1 to about 50 Angstroms; and C is defined as an optionally substituted azobenzene group. In particular invention embodiments, A is maleimide and C is azobenzene. In a particular invention example, B is further defined as a C_1 to C_{50} alkyl group, alkyl polyol, polyoxyalkyl group, polymethylol, or polyoxyethylene group. A specifically preferred bifunctional linker is shown in Figure 4.

As discussed, in some invention embodiments the polyfunctional linker will be a dendrimer. Preferably, that dendrimer is represented by the following Formula II:

$(P)_X^* (M)_Y$

Formula II

in which P is defined as a dendrimer, X represents an integer of 1 or greater, each M represents a receptor according to the invention, y represents an integer of 1 or greater; and

* indicates that the receptor is associated with the dendrimer either covalently, non-covalently, or a combination thereof. Examples of more preferred dendrimers include those having starburst, dense star, rod-shaped and related configurations suitable for association with the receptors and semi-conductive films described herein have been reported in U.S. Pat. Nos. 5,338,532, 4,694,064; 5,527,524; and 5,788,989; the disclosures of which are incorporated herein by reference.

Additionally preferred dendrimers in accord with the invention have a diameter of from between about 10 Angstroms to about 5000 Angstroms, preferably 100 Angstroms to about 1000 Angstroms.

Also preferred is a dendrimer which, prior to reaction with the receptor and the semi-conductive film, has at least one electrophilic group on a first end of the dendrimer and at least one photoactivatable group on a second end of the dendrimer. In this invention embodiment, the receptor will often, but not exclusively, be non-covalently attached to the electrophilic group on the first end of the dendrimer. Preferably, the photoactivatable second end of the dendrimer is further bound to the polymer material. Such a dendrimer can be bound to the backbone of the polymer material in nearly any acceptable way including covalently or non-covalently with covalent attachment being preferred for most applications.

In invention embodiments in which the dendrimer has at least one electrophilic group, that group is typically polar. Dendrimers with at least one photoactivatable group are especially well suited for use with this invention. In this

illustration of the invention, the photoactivatable group is usually, but not exclusively, hydrophobic.

In one sensor embodiment, an appropriately selected receptor is combined with a suitable polyfunctional linker to form a complex. As discussed, the polyfunctional linker can be eg., a bifunctional or dendrimeric linker. Such a complex can, for example, be immobilized by an appropriate chemical or photochemcial reaction with the semi-conductive film and more specifically with the polymer material of that film. Polymerization of the film polymer material can be effected before, during, or after reaction with the receptor-polyfunctional linker complex with reaction after the polymerization being preferred for many uses of the invention. In addition, such polymerization can be achieved before, during or after reaction of the polyfunctional linker with the film in which case a suitable receptor will be bound to the linker before, during of after binding between the semi-conductive film and the polyfunctional linker. More specific methods for combining these components of the sensor are provided below. Nearly any effective means for performing the polymerization can be used with this invention, particularly those which are optimal for polymerizing materials that include or consist of a suitably conductive epoxy resin, polymer, co-polymer, graft co-polymer, or polymer alloy.

Particular conductive and semi-conductive polymers suitable for use with this invention have attracted much interest. For example, there is recognition that organic polymers, like metals and inorganic semiconductors, can be made to support transport of considerable electrical charge. The earliest studies were performed with polyacetylene, among the simplest of all of the conducting polymers. Since then, conductive polymers such as polyanaline, polythiophene, and polypyrrole have found application as artificial muscles, transparent electrodes, variable-tint "smart" windows, solar cells, light-emitting diodes, electrostatic discharge films, electromagnetic interference shielding, and most notably, as chemical and biological agent sensors.

Conducting polymers are generally characterized by an alternating sequence of single and double bonds along the polymer backbone. At the electronic level, this

alternating bond sequence, or conjugation, is believed to help formation of energy "bands"—molecular orbitals through which electrons can travel, much like in metals. On exposure to chemical or biological species, the relative distribution of electrons within these "bands" can change. Such distribution changes may be manifest as changes in the optical properties (absorbance or fluorescence) or electrochemical properties (oxidation/ reduction potentials) of the conductive polymer. Electrical conductivity changes can also occur. Conductivity in conductive polymer films has largely two components: conductivity due to electrons traveling along the backbone of the individual polymer chains (intrinsic conductivity), and that due to electrons "hopping" between polymer chains (extrinsic conductivity). While a polymer's extrinsic conductivity is generally several orders of magnitude smaller than its intrinsic conductivity, modulation of both types can be exploited in sensing applications.

Without wishing to be bound by any theory, it is believed that many conductive polymer sensors rely on changes in extrinsic conductivity that occur when a conductive film is swollen by small chemical species, for example. As the polymer chains are pushed apart by the ingressing molecules, the electrical resistance changes (Figure 18). The intrinsic conductivity of a polymer film is sensitive to chemical reactions between reactive analytes and individual polymer chains. For example, acids and bases effect increases and decreases in polymer conductivity, respectively. Mechanistically, modifications to the chemical/ electronic structure of the individual polymer chains has a larger effect on overall film conductivity than do changes in the chains' distance from one another.

Although it is believed that mechanical perturbation of individual chains may play a role in helping conductivity changes, it is stressed that such perturbation may not be the only means of generating such changes according to the invention. In particular, conjugated systems are characterized by spatial overlap of the π-orbitals on adjacent repeating units of the polymer. Conjugation along the polymer backbone is effectively "broken" wherever the repeating units are not coplanar and cannot overlap. While conducting polymer chains are reported to be fairly rigid, they are believed to have twists and kinks along their length. There is understanding that the

electrons will travel along the "unbroken" conjugated system until they reach a "break" (twist or kink), at which point they must "hop" to a neighboring chain. The present invention makes use of this phenomenon to detect biologically-active species like bacteria, viruses, etc.

Many of the analytes detected by the implementations of this invention are thought to be too large to diffuse into the conductive polymer film. Without wishing to be bound to any theory, it is believed that such diffusion of small analytes helps to push the polymer chains apart (affecting extrinsic conductivity) or engage in chemical reactions with polymer chains in the bulk of the film (affecting intrinsic conductivity). It is believed that good detection according to the invention is helped by interactions with the analyte and the conductive film surface, and these interactions are mediated by the presence of film surface-bound biospecific ligands. When these ligands bind to the analyte, it is believed that in many instances the individual polymer chains at the conductive film surface are mechanically-perturbed. It is this perturbation that is thought to facilitate measurable changes in the film's electrical resistance, indicating the presence of the analytes of interest.

A wide spectrum of polymer materials can be used in accord with this invention. Such acceptable materials generally include a plurality of conjugated carbon atom bonds eg., an optionally substituted polyanaline, polythiophene, polyacetylene, polypyrrole, polyparaphenylene, or polyparaphenylene vinylene polymer. As an example, polymerization of such species can be initiated chemically, thermally, or photochemically by addition of an appropriate catalyst, initiator or crosslinking agent, e.g, glutaric dialdehdye (glutaraldehyde), heat, or ultraviolet light, to at least the monomer of the intended polymer, , preferably in combination with the polyfunctional linker. Other suitable materials which can be used to polymerize the polymer material will depend e.g., on the compounds selected and can include SPDP, dimethyl suberimidate, disuccinimidyl suberate, bismal bimidonexane and the like.

Choice of a suitable receptor will be guided by ability to bind a particular analyte or group of analytes for which detection in a given sample is desired. As used

herein, the term analyte of interest refers to an individual analyte of interest or a specific class (or type) of analyte bound by the receptor.

In another embodiment of the invention, the polymer material further includes at least one chemical group adapted to join the second end of the polyfunctional linker to the polymer material. Other suitable polymer materials according to the invention include those featuring an electrical conductivity between from about 10^{-16} to about 10^{-3} $ohm^{-1}.cm^{-1}$ and/or a thermal stability of up to about 200°C as determined by thermal gravimetric analysis.

As discussed, the invention is compatible with use of a wide range of semi-conductive films. Included in such films are those having at least one additive or a component for increasing or decreasing electrical conductivity. An exemplary additive is an optionally substituted lower alkyl diamine, preferably hexanediamine. Preferably, the additive is present in the polymer material in an amount that is conducive to acceptable signal transduction and good signal output.

Examples of suitable components for inclusion in the semi-conductive films of this invention include at least one of a metal, metallic alloy, or a carbon material. Preferably, those components are present in an amount that is conducive to acceptable signal transduction and good signal output. Preferred metals include gold, silver, copper, platinum, and nickel. Preferred carbon materials include carbon black, graphite or a carbon nanotube.

The polyfunctional linkers of this invention including those covalently or non-covalently bound to one or more receptors can be configured in the sensor in nearly any suitable arrangement such that the implementation and more specifically the senor, biosensor, or bioaffinity sensor can detect and optionally quantify the analyte for which it was intended. As discussed, sensors having particularly dense linker networks are often preferred. In this illustration of the invention, each sensor in the array includes a plurality of polyfunctional linkers having a density of between from about $0.1x10^{11}$ to about $25\ x10^{12}$, preferably about $3x10^{11}$ to about $8x10^{12}$ as determined by atomic force microscopy.

In those invention embodiments in which especially dense linker networks are preferred those networks will sometimes be referred to herein as an aggregate. Preferably, each aggregate has a length of between from about 1 nm to about 500 nm, preferably about 5 nm to about 100 nm.

Preferred receptors according to the invention are generally capable of binding at least one analyte (or class of analytes). Typically a receptor of interest will bind about one analyte which binding can be specific or non-specific as needed.

By the term "specific binding" or similar term is meant a molecule disclosed herein which binds another molecule, thereby forming a specific binding pair, but which does not recognize and bind to other molecules as determined by, e.g., Western blotting, ELISA, RIA, gel mobility shift assay, enzyme immunoassay, competitive assays, saturation assays or other suitable protein binding assays known in the field.. By the term "non-specific" binding is meant capacity to bind more than one analyte (or class of analyte) as determined by standard assays such as radioimmunoassay (RIA), Western blot, ELISA test, ect.

See generally Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, New York (1989), and Harlow and Lane in *Antibodies: A Laboratory Manual,* CSH Publications, N.Y. (1988), for disclosure relating to the methods for detecting specific and non-specific binding.

Illustrative receptors include nucleic acids (DNA, RNA or DNA/RNA hybrids) as well as synthetic or semi-synthetic derivatives thereof (e.g., nucleic acids including radionuclides or sulfur atoms substituted for oxygen in the phosphodiester linkage). Additional receptors of interest include amino acid sequences having D and/or L amino acids including peptides, polypeptides, proteins. Also envisioned are lipids, carbohydrates, lectins, glycoproteins, glycolipids and the like. More specific peptides, polypeptides and proteins of interest include biological or modified derivatives of same including methylated, acetylated, myristylated and phosphorylated protein and polypeptide sequences. Additionally specific proteins

include antibodies as well as antigen-binding fragments thereof such as $f(ab')_2$ and Fab fragments. Also envisioned are enzymes, preferably surface modified enzymes, as well as substrate binding fragments thereof. Also contemplated are nucleic acid receptors that include or consist of catalytic DNA or RNA.

See also U.S. Pat. Nos. 4,562,157; 5,766,934; 5,001,048; 5,192,507; and 5,352,574 for disclosure relating to other suitable receptors including enzymes, polyclonal antibodies, monoclonal antibodies, cell membrane receptors, lectin, antigen, ect.

As mentioned, the invention is compatible with the detection of a wide spectrum of analytes. Such analytes include, but are not limited to, those analytes which are naturally-occuring, synthetic, semi-synthetic or a result of forced evolution.

Particular examples of natural ligands include nucleic acid (RNA, DNA, or RNA/DNA hybrid), peptide, polypeptide, protein, carbohydrate, enzyme substrate, lipid, fungus, glycolipid, glycoprotein, antigen, virus, prion, metazoan cell; or receptor binding fragment thereof. More specific peptide, polypeptide and protein analytes include biological or modified derivatives of same including methylated, acetylated, myristylated and phosphorylated protein and polypeptide sequences.

Examples of forced evolution analytes include recombinant carbohydrates, nucleic acids (RNA, DNA), antibodies, and peptides. Examples of synthetic analytes include proteins, peptides, antibodies, and nucleic acids made whole or in part by the use of an automated or semi-automated device for manufacturing same.

More specific analytes of interest include hormones, vitamins, cytokines such as the interleukins, cytotoxins such as bacterial toxins, blood factors such as Factor X, growth factors such as human growth factor, environmental toxins such as heavy metals and organometallic compounds. Additionally specific analytes include primate parasites and especially bacterial, helminthic (nemotodes, tapeworms, flukes) and protozoan (malaria, leishmaniasis, giardiasis, amebiasis, trypanosomiasis, ect.) cells

including spirochetes. More specific cells of interest are those associated with human disease or discomfort.

Examples of preferred cell analytes include those bacteria associated with food borne diseases (eg., Campylobact. and Salmonella); Legionnella, Tuberculosis, sexually transmitted diseases (STDs such as Chlamydia, and Gonorrhea). Additionally preferred analytes include viruses such as those associated with immune system dysfunction (HIV), childhood illnesses (chicken pox, epstein barr virus), influenza, herpes (including those responsible for genital warts and cold sores). Also preferred are protein analytes such as those associated with allergies including those of animal (domesticated animals such as cats and dogs), fungal and plant origin.

Other exemplary cell and viral analytes have been reported by Sande, MA and Mandell, G.L. in Chemotherapy of Microbial Diseases, *The Pharmacological Basis of Therapeutics,* pp. 1066-1094 MacMillan Publishing Co. (New York) (1985); the disclosure of which is incorporated herein by reference.

As discussed, the sensors of this invention can be used to analyte samples in a variety of forms such as a liquid, gas, vapor, mist or an emulsion. In some embodiments, the sensor can be used in instances in which the liquid is a flow stream. In such an embodiment, the sensors described herein have several applications including use in the monitoring of industrial effluents and quality control analysis.

The implementations of the present invention including specified sensors can be operably linked to one or more additional components which components can be integrated as part of the implementation. Alternatively, or in addition, such components can be present in a stand alone configuration. Selection of a specific implementation format will often be guided by intended use such as whether a sensor is to be used in the field (in which case stand alone component configurations may be suitable) or in a laboratory setting.

For example, in one invention embodiment, the implementation further comprises a pump operably linked to the implementation. In another embodiment, the

implementation further comprises a computational system operably linked to the detector for manipulating the output. In this example, output is preferably stored by the computational system and optionally processed prior to display to the user. Acceptable manipulations include comparing the output to a standard analyte concentration curve and determining the amount of analyte in the sample. Typically, the output is displayed to the user essentially in real-time.

In certain invention embodiments, it may be useful to include a control sensor which sensor can register implementation changes unrelated to analyte binding. As an example, such a control sensor can include essentially the same components of the test sensor with the proviso that the control sensor not include the receptor.

Other preferred implementations include those capable of remote detection of an analyte as well as those suitable for wireless communication system.

In a more specific embodiment, the implementation includes at least one and preferably all of the following components:

a. a sensor array comprising one or a plurality of sensors in which each sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber having dimensions less than about 10 nm x 10 nm,

ii) a semi-conductive film having a thickness of between from about 50 Angstroms to about 100 Angstroms and positioned at least in the chamber, the film providing electrical contact between the electrodes; and either

iii) a bifunctional linker having a length of from between about 2 nm to 10 nm and comprising a first end attached to a protein, nucleic acid; or analyte binding fragment thereof, the linker further comprising a second end, or

iv) a dendrimer having a diameter of from between about 2 Angstroms to about 10 Angstroms and comprising a first

end attached to the protein, nucleic acid; or analyte binding fragment.

In a more preferred implementation, the dendrimer further includes a second end, in which the semi-conductive film includes at least one of an optionally substituted polyanaline, polyparaphenylene, polyacetylene, polyparaphenylene vinylene, or polythiophene polymer. Preferably, the second end of the bifunctional linker or dendrimeric linker is attached to the polymer, the film being capable of registering analyte binding to the protein, nucleic acid; or fragment thereof as a change in electrical conductivity of the film. That change is taken to be indicative of the presence of the analyte in the sample.

As discussed, the present invention features sensors including particular biosensors and bioaffinity sensors (sometimes also called bioaffinity biosensors herein). Thus, in one aspect, the invention discloses bioaffinity biosensors that include at least one type of modified conducting polymer (sometimes called semi-conductive polymer), usually one of such polymers.

To make a specific bioaffinity biosensor according to the invention, a particular conducting polymer (e.g., polyaniline, polypyrrole, polythiophene, etc.) can be deposited as a film into the narrow channel between two electrodes on an insulating substrate, so as to close what would ordinarily be an open electrical circuit. In some embodiments, the electrodes are interdigitated. The conducting polymers are preferably modified with at least one type of biological macromolecular receptor (sometimes called a ligand). As discussed previously, such receptors include but not limited to proteins (including antibodies and antigen-binding fragments thereof; lectins, antigens, cell membrane receptors such as the acetylcholine (AChR) receptor or ligand binding part thereof), nucleic acids and lipids, as well as synthetically-modified materials from both natural and synthetic sources.

A receptor of interest is attached to the polymer films by one or a combination of different approaches. In one approach, the ligands are first coupled to a reactive agent. The linkage connecting the ligand to this agent may be a covalent bond, non-

covalent bond (using through-space hydrogen and/or van der Waals forces), or ionic bond. The same or different chemistries may be used to then attach the ligand-reactive agent conjugate to the surface of the conducting film, or the ligand may simply be blended into the polymer prior to film deposition.

Figure 1 shows binding of a specific biomacromolecular analyte (SA, streptavidin alkaline phosphotase) to a particular ligand-modified conductive polymer film (streptavidin-specific bioreceptor). As shown, specific binding between the SA-peptide and the bioreceptor effects changes in the electrical resistance of the polymer. See also Figure 2 (showing a clear drop in electrical resistance on exposure to the biomolecular target).

The following discussion relates to more specific sensors of the invention.

A. Real-time Sensor For Rapid Diagnosis of Mycobacterium TB

Zoonoses, or infections acquired from animals, affect both animal care workers and experimental animal populations, and are well-recognized risks in research animal facilities. It has been established that Non-Human Primates (NHPs) can be a source of a variety pathogens. For example, infection with Mycobacterium tuberculosis (TB) can result from contact with infectious aerosols produced by experimentally- and naturally-infected animals. TB is a major international human and animal health issue and screening for this disease can be problematic and expensive.

TB infection is defined as a small number of TB bacteria living in a host but not causing illness. This occurs when TB bacteria are inhaled into the tiny air sacs of the lungs. A host with TB infection does not feel ill, has no symptoms, and cannot spread TB bacteria to others. If the host's immune system becomes weak, however, TB infection can develop into TB disease. Individuals with TB infection are at risk of developing TB disease throughout their lifetime unless properly treated.

NHPs that have been bred and maintained in a well-controlled environment have had little opportunity to contract unwanted infections. Other animals coming from sources that are less regulated and less stable have more opportunity to acquire an infection which can subsequently be introduced into the animal care facility. Due to the time and expense involved with breeding NHPs to a point where they are sufficiently mature to act as a model for human disease, many researchers have no choice but to utilize animals from uncontrolled sources, often the wild.

The challenges in the detection of TB relate to not only the aerosol route of infection, but also to the general expense of the process. Detecting the disease in individuals who show clinical signs (because of high titer of the organism) is not a difficult task. However, TB can exist in a carrier state with intermittent shedding of the organism. This situation requires multiple cultures on a given animal to determine whether the animal is infected. With a laboratory culture costing up to fifty dollars per test, the process becomes prohibitively expensive.

The availability of a real-time detector for TB organisms would offer animal care facilities a cost-effective mechanism for protecting the health of animal care workers and the experimental populations in their charge.

Accordingly, the invention features a real-time sensor for Mycobacterium tuberculosis (the causative agent for tuberculosis in non-human primate populations) that exhibits extremely high sensitivity. In order to achivev the level of sensitivity desired, it is an objective of the invention to adapt the ligand-CP transduction method described herein. To this end, an innovative scheme that significantly increases the density of bioconjugates (biospecific ligand + linker arm) attached to the CP film surface is proposed. Preliminary experiments made use of water soluble, photoactive bioconjugates. These bioconjugates were covalently attached to the film by UV irradiation of dilute bioconjugate solutions in which the film was immersed. Using this approach, the maximum ligand surface density achievable was limited by (1) the bioconjugate solution concentration (i.e., the rate at which the bioconjugates diffused to the film surface), (2) the probability that the photoactive termini of the bioconjugates actually attached to the surface (rather than to other surface-bound

ligands), and (3) the steric constraints imposed by the bioconjugate's hydrodynamic volume (the amount of space each surface-attached bioconjugate requires at thermodynamic equilibrium). The proposed approach addresses all three of these limitations simultaneously. First, artificial packing of the bioconjugates together in a prescribed orientation and at high density will be achieved. Subsequently, attachment will be initiated photochemically of the ordered aggregates to the CP film. This is described in detail below.

As mentioned above, the bioconjugate consists of a photoactive base and a bioactive ligand. The base will be processed to form a densely-packed array (i.e., like a thick "lawn") covalently bonded to the surface of the conducting polymer film by simple irradiation with UV light. Following the conjugation of the base to the film surface, electrophilic termini on the base will be covalently bound to the ligand using standard solution chemistry. In this novel approach, it is also possible to pre-form the bioconjugate (including both base and bioactive ligand), then covalently bond it to the surface of the conductive polymer film. The ligand may be an antibody or antigen-binding fragment thereof that will bind irreversibly to a specific analyte. With regard to relative sizes, the base will be approximately 5 A across by 25 A long, and the antibody fragment will be approximately 45 A across. In contrast, the M. tuberculosis bacterium is approximately 0.1 -1 1lm in diameter. Upon binding, the conformation of the ligand and the base will change, causing a change in the conformation of the surface of the conducting polymer, as we have previously shown. The densely-packed bioconjugate array should afford a 1 to 2 order of magnitude amplification of the signal due to the larger disruption of the conformation of the conductive films. This is schematically represented in Figures 3A and 3B.

B. Real-time Sensor For Rapid Diagnosis of Pediatric S. Pneumoniae and Varicella

The present invention encomapasses a unique approach to sense and detect the specific pathogens responsible for Pediatric S. Pneumoniae and Varicella. In one embodiment, the inventioin provides for real time diagnosis of these diseases and

offers significant cost savings to the health care industry in identifying and treating these conditions. The proposed sensors will obviate the use of time-consuming laboratory tests and will offer the clinician the opportunity to prescribe infection-specific drugs.

In cases in which an infant patient presents with a Fever Without Source (FWS) or Fever of Unknown Origin (FUO), a number of infectious agents such as bacteria or viruses could be the cause. Rapid and specific diagnosis of these pathogens will result in a significant improvement in the quality of care received by the child.

To detect pathogens associated with these diseases, the invention featurs a particular pediatric biosensor. That sensor has many commercial medical applications for point of care diagnosis of pneumonia and chicken pox. It will be appreciated that such a pediatric biosensor can be used for the adult population as well. This basic technology behind the proposed sensors has many applications in detecting and quantifying bacterial and viral pathogens of any kind.

The challenges for the clinician engaged in the diagnosis of the pediatric patient are numerous. In cases in which an infant patient presents with a Fever without source (FWS) or Fever of unknown origin (FUO), rapid and specific diagnostics and therapies would be preferred. However, the required time and physical complexity of obtaining sufficient bodily fluids for culture and identification, and the incubation period required for bacteriological identification of an infectious agent have precipitated the frequent and prophylactic use of broad spectrum antibiotics. Even before a diagnosis is made antibiotics are prescribed. The rational for this approach is commonly that "time is the enemy". Particularly with patients who cannot communicate the specifics of their discomfort or potential exposure, early intervention on a broad scale is preferential to a laboratory-based diagnosis due to the fragility of the patient. The risks with the first exposure of a young patient to a broad spectrum antibiotic are that a drug reaction, allergic reaction, or hypersensitivity may occur. Additionally, if the patient is presenting in an urgent situation, intravenous introduction of antibiotics will likely precede oral introduction and may exacerbate an

adverse response. Overuse of broad spectrum antibiotics has also been implicated in the increased observation of a number of antibiotic resistant organisms.

Time is one of the most critical elements in diagnostic-therapeutic cascade, both in urgent and scheduled medical care situations. In cases where FWO and FWS present, a number of infectious agents such as bacteria or viruses could be the cause. Standard diagnostic protocol dictates the procurement of samples from the patient for bacteriological examination. This examination involves culturing the samples in order to obtain sufficient numbers of infectious agent to allow identification. A prescribed period of time is required for the identification process to be completed. In some cases, two weeks pass before laboratory results are available. In cases that present in the clinic, samples are delivered to the in-house laboratory. However, with samples obtained in the offices of private physicians, a transportation protocol must be followed to get the samples out of the office, to the hospital or commercial lab where the tests are performed.

These problems highlight the urgent need a diagnostic tool that will identify specific infectious agents, both viral and bacterial, in real time, in the clinician's office, without the expense and time delay of a laboratory-based assay. Rapid identification of infectious agents will allow clinicians to tailor specific therapeutic solutions to meet the immediate needs of pediatric patients.

The present invention proposes, in one embodiment, a unique approach to the sensing and detection of the specific pathogens that are responsible for Pediatric S. Pneumoniae and Varicella. This inventive concept allows real time diagnosis of these diseases and offers significant cost savings to the health care industry in identifying and treating these conditions. The proposed sensors will obviate the use of time-consuming laboratory tests and will offer the clinician the opportunity to prescribe infection specific drugs.

More specifically, a more sensitive biosensor is proposed. In a particular embodiment, the biosensor will employ linkers intercalated in the bulk of the CP film to amplify the perturbation registered in the film by the binding event. Development

of a sensor that would be specifically used for identifying and quantifying pediatric pneumonia and chicken pox is an objective of this invention. Of course, the resulting sensor can also be used for the diagnosis of these diseases in the adult population. The details of the proposed concept are described in the following paragraphs.

In the traditional construction of a conducting polymer film-based biosensor, a bioligand is either intimately mixed into the conducting polymer matrix or is tethered to the film surface. The most popular mode of action involves conversion of the target species to some electroactive product that then diffuses into and reacts with the underlying polymer film layer. After reacting with this electroactive species, the polymer exhibits a measureable change in conductivity (i.e., electrical resistance). For example, organo phosphohydrolases (OPH) will hydrolyze agents (such as the insecticide paraoxon, or the nerve agent sarin) to phosphoric acid, which may then be detected by measuring the change in resistance in the conductive polymer film. It is not intuitively obvious, however, that the effect of a simple bioligand/target binding event (which causes a conformational change in the bioligand) could be transferred to the underlying polymer. In fact, it has been demonstrated that the binding of an antibody (attached to a conducting polymer film directly or through a short spacer arm) does not generate a detectable signal due to the rigid structure of the antibody. (That is, minimal conformational change occurs in the antibody on binding). However, preliminary experiments have indicated that using a similarly-rigid bioreceptor system, a significant change in the CP's electrical resistance can indeed be generated. It is believed that the resistnace change generated by a simple binding event between target molecule (or cell) and bioligand (antibody) may be amplified by intercalating the spacer arm into the conducting polymer, rather than just attaching it to the film surface.

To accomplish this, use previously reported techniques to synthesize amphiphilic "rod-coil" triblock macromolecules is proposed. These species consist of a rigid, "rod"-like segment, and a flexible "coiled" segment. As described in the literature, rationally-designed rod-coil macromolecules form a thermodynamically-favored nanostructure in which the rod-like segments self-assemble into a closely packed array, while the coil-like segments segregate into

their own nanophase. The resulting "mushroom" shape and the dimensions of these nanostructures are shown in Figures 8A and 8B. It should be noted that in the same way that the individual rod-coil molecules are amphiphilic, so the aggregated nanostructure is also amphiphilic, with the bundled rod-like structures being hydrophilic and the coil-like segments being hydrophobic (or vice versa).

Amphiphilic molecules tend to align themselves in hydrophobic or hydrophilic media so as to realize the most energetically-favorable orientation. That is, in aqueous Langmuir-Blodgett layer-by-layer film fabrication, for example amphiphilic species orient themselves at the air-water interface so that their hydrophobic ends protrude out of the water, while their hydrophilic ends remain immersed in the aqueous subphase. Similarly, and in accord with this invention, it is proposed that the hydrophilic rod-like stalks of the "mushroom" nanostructures will remain immersed in an aqueous solution of conducting polymer (or polymerizable monomer thereof), while the hydrophobic coil-like "caps" will protrude above the liquid surface into the air. The nanostructure may be self-assembled by dispersing the individual rod-coil macromolecules in cyclohexane, allowing them to aggregate into the "mushroom" structures, then adding the organic phase to the surface of the aqueous polymer solution. The cyclohexane can then be removed in vacuo, after which the aggregates will orient themselves as just described, forming a thin film at the air-water interface. After removal of the water, the rod-like stalks of the "mushrooms" will remain intercalated into the conductive polymer film. The bioligands are then attached to the electrophilic maleimide end group of the coil.

Since the target biologicals in this example of the invention are the causitive bacteria for pediatric pneumonia and the viral agent for chicken pox, which are much larger than the antibody bioligands, the antibodies will bind to these targets at multiple sites on the coil-like "mushroom" segment above the conducting polymer. (The dimensions bacteria and viruses are approximately 1000 to 5000 nm and approximately 10 to 50 nm, respectively). A bacterial cell, in particular, will also not simply bind to one "mushroom" structure, but rather to many. The effect will be to exert force on several individual "mushrooms" at once, each of which will have several attachment points to the underlying conductive polymer (rather than one

single attachment point, like the spacer arms we employed in our preliminary work). The result will be a very pronounced change in the polymer's conductivity. As these coils separate to accommodate the presence of the large analyte species, they will induce a "tilting transition" in the rod-like stalk (embedded in the conducting polymer) to relieve the stress. In the "tilting transition", the individual rod-like portions of the macromolecules within the "mushroom" slide against each other and will cause a conformational change in the conducting polymer. This conformational change will then give rise to a change in the polymer's electrical resistance.

Based on this concept, a biosensor can be designed that is specific to the pathogen in question and can both identify and quantify it. Such a biosensor has many commercial medical applications for point of care diagnosis of pneumonia and chicken pox. Though this effort is geared toward pediatric sensors, the resulting sensors can be used for the adult population as well. This basic technology behind the proposed sensors has many applications in detecting and quantifying bacterial and viral pathogens of any kind.

C. Real-time Sensor For Rapid Diagnosis of Food Poisoning Pathogens

The present invention also contemplates real-time surveillance of foodborne pathogens. This feature of the invention offers significant cost savings to the food processing industry and hospitals in preventing, identifying, and treating food poisoning cases. The proposed sensor will facilitate the monitoring of food facilities and offers a lowcost, real-time alternative to current clinical diagnostic methodologies. Each year, foodborne illness in the United States causes over 20 million cases of human illness resulting in 10,000 annual deaths and a financial impact estimated at tens of billion dollars.

More particularly, the invention provides a unique approach for the detection of specific pathogens responsible for a significant portion of foodborne illnesses (e.g. Salmonella Enteritides and E. coli).

One approach according to the invention relies on providing a good transduction mechanism for detecting biomolecules. The proposed concept builds on

these developments to develop a second generation, inexpensive, yet accurate biosensor that can identify and quantify pathogens in real time. Foodborne illness in the United States is a major cause of personal distress, social disruption, preventable death and avoidable economic burden. As identified and described by the Massachusetts Department of Public Health Working Group on Foodborne Illness Control, a leading agency in epidemiologic investigation, the challenge of managing foodborne pathogenesis is a wide-ranging activity spanning a breadth of disease states and mechanisms of infection. In the late 1990's, foodborne diseases caused an estimated 24 to 81 million sporadic and outbreak-associated cases of human illness and 10,000 deaths annually in the United States. The economic impact of illness at this scale is staggering since the unpleasant symptoms of even a mild case of foodborne illness may require absence from school or work. Some investigators estimate that the financial impact of diarrhea foodborne illnesses is between $7 and $17 billion per year in the United States alone.

The majority of foodborne diseases are caused by microbial pathogens such as viruses, bacteria and parasites. One way of categorizing foodborne illness is by the mechanism by which it is inititated: a) foodborne infection, which occurs when the infectious organism is ingested and invades and multiplies in the victim's intestinal lining, or b) foodborne intoxication, where an organism produces a toxin in food that is subsequently ingested. Ingested pathogens, transmitted from contaminated foods, enter the body by way of the gastrointestinal (GI) tract. The healthy human body has defenses to fight these pathogens, but an overwhelming dose of pathogens or a weakened resistance can lead to illness. Certain populations (for example, the very young, the elderly, and some immunocompromised persons) are at higher risk for foodborne disease and for serious complications of foodborne disease. The severity of illness may be different among people eating the same contaminated food. The variability in illness severity is due to several factors, including: the concentration of the pathogen, the virulence of the pathogen, and the health status of the host.

Currently, approaches to the detection and identification of pathogens that cause foodborne diseases in food production and preparation facilities include both scheduled and unannounced testing for the presence of harmful organisms. This

involves gaining physical access to food production, preparation, inspection, packaging and distribution facilities, and sampling the environment with swabs, open plates and other techniques to obtain samples for cell culture. Once obtained, cultures are plated on a variety of media and allowed to grow. Once there are sufficient numbers of organisms to examine, and the culture has not become contaminated, a biochemical analysis is initiated. The cultures are maintained and observed in some cases up to 14 days. During that time cultured organisms are identified and classified. If during the course of the culture and identification process, a pathogen is discovered a prescribed plan of action is implemented. This forensic approach (on-site sampling + pathogen isolation + 2-week lab culture + identification/ classification) requires a lengthy culture period for the growth of the organism that results in an "unprotected period" between potential pathogen sampling and final bacteriological identification. That is, the time constraints dictated by culture-based diagnostics could result in the release of contaminated food products because in many cases food products are not held in quarantine pending the results of facilities testing.

Once an individual has contracted a foodborne disease, an immediate clinical solution is often demanded. Emergent care includes the evacuation of the GI tract, hydration, sampling for bacteriological identification of the infecting agent, and remediation of the causative agent. As indicated earlier, this process requires significant financial overhead and is painfully slow in the light of clinical urgency.

The traditional culture-based bacteriological analyses have been shown to be a reliable approach for contaminant detection. However, the high cost of maintaining and supplying the laboratories and supporting highly trained staff, in addition to the time required between sampling and pathogen identification suggest that this process could benefit from a real time, organism-specific identification system.

The present invention features a unique approach to the sensing and detection of specific pathogens that are responsible for a significant portion of foodborne illnesses (such as Salmonella enteritidis and Escherichia cold 0157:H7). In one embodiment, the sensor allows real time surveillance of foodborne pathogens and offers significant cost savings to the food processing industry and hospitals in

preventing, identifying, and treating food poisoning cases. This surveillance may take place at various points on the chain of food processing and preparation events, as well as at the site of clinical care for a foodborne illness. The proposed sensor facilitates the monitoring of food facilities and workers and offers a low-cost, real-time alternative to current clinical diagnostic methodologies.

More specifically, the invention features a more sensitive biosensor to detect these cells. One proposed approach uses dendrimer technology to amplify the perturbation registered in the CP film by the binding event. It is specifically proposed to make a sensor that would be specifically used for identifying and quantifying food poisoning pathogens.

Preliminary data demonstrate that the binding of a large biomolecule to a suitable bioligand will change the electrical resistance of the conductive polymer film to which the ligand is attached. This scheme obviates the need for generation of a secondary electroactive moiety that must diffuse into the conducting polymer to elicit an effect, as in enzyme-based glucose sensors. In order to amplify the direct-transduction effect, the invention features use of highly-branched dendrimers as ligand linkers. As described previously, dendrimers have well-defined architectures, and thereby can covalently attach to the conductive polymer film at many different points, causing greater mechanical perturbation of the film when the analyte binds to the ligand. The nascent construction of dendrimers will permit the synthesis of structures that contain two distinct surfaces. One surface will be activated to covalently bond to the conducting polymer surface and the other, when coupled to the bioligand, will interact with food poisoning bacteria. The surface that will bond with the conducting polymer surface will be photoactive. The surface that will bind to the target cell will use a prebonded IgG antibody fragment against surface epitopes of the target.

Bacterial cells are relatively large (0.2 to 0.6 ,um in diameter), while the dendrimers are smaller nanostructures that may have diameters of approximately 0.01 to 0.1 ,um. Consequently, a bacterial cell will not simply bind to one dendritic macromolecule, but rather to five or ten. The cell will then exert force on several

individual dendrimers at once, each of which have several attachment points to the underlying conductive polymer (rather than having only one single attachment point, like the linkers we used in our preliminary experiments). This is shown schematically in Figures 12A and 1B. The result will be a very pronounced change in the polymer's conductivity.

It will be apparent that the proposed biosensor has applications in monitoring food manufacturing lines as well as point of use diagnosis of pathogens responsible for foodborne diseases. This integrated system has dual use applications as a medical diagnostic tool (e.g., for screening biopsied tissue samples), or for maintaining supermarket/restaurant/ cafeteria quality control (e.g., spoilage/contamination assessment).

As discussed, the invention provides specific biosensor for detecting a peptide that binds streptavidin. In one example of such a sensor, the peptide is combined with an (*N*-hydroxysulfosuccimidyl-4-azidobenzoate) linker which linker is combined with a suitable semi-conductive film such as polypyrole under conditions that activate the azidobenzoate group to effect covalent bonding thereto. Generally, but not exclusively prior to such photoactivation, the film was subjective to known conditions suitable for polymerizing the film. In most cases, such polymerization is achieved by mixing and casting monomer between the set of electrodes, generally within a few minutes after initiating polymerization. The result is a semi-conductive film. As discussed througout this application, such a film can be may "layer-by-layer" which has been found to produce exceptionally sensitive sensors. If desired, the coated semi-conductive film can be aged for a sufficient time, usually about a few hours up to a day or so.

The streptavidin sensor just discussed (sometimes also called a streptavidin-alkaline sensor) is described more specifically in Example 1 below. Such a sensor can be used to test the functionality of nearly any sensor component including a desired semi-conductive film, receptor and polyfunctional linker. Particular combinations of such films, receptors and linkers can also be conveniently tested. Importantly, such a sensor can be used to select or modify those components

(receptor, polyfunctional linker, polymer) having one or a group of desired performance characteristics.

Testing a polyfunctional linker. Referring now to Example 1 below, the (*N*-hydroxysulfosuccimidyl-4-azidobenzoate) linker of the standard streptavidin sensor can be replaced with a test polyfunctional linker preferably also having the azidobenzoate end group. The test linker can then be covalently bound to the peptide as described. This conjugate can be added to the surface of a polypyrrole (or other suitable conducting polymer as described herein) film and subjected to UV irradiation to induce covalent bonding to the film. After the ligand-functionalized film equilibrated somewhat in glass-distilled deionized water, the film can be exposed to a solution of Streptavidin-Alkaline Phosphatase (SA). The test polyfunctional linker is "acceptable" if it facilitates at least about a 20% decrease in the resistancepreferably at least a 50% decrease and more preferably from about 75% to about 95% or more of the resistance decrease seen with the standard streptavidin biosensor.

Testing other sensor components. It will be apparent that other components of the foregoing streptavidin biosensor can be used to test other components such as the type of conductive film including films having two or more conductive polymers. Also, the addition of various polymer additives and components can be evaluated by using this biosensor. A preferred conductive film, additive, or film component is acceptable if it facilitates at least about a 20% decrease in the resistance, preferably at least a 50% decrease and more preferably from about 75% to about 95% or more of the resistance decrease seen with the standard streptavidin biosensor.

A variety of receptors suitable for use with this invention including particular antibodies and cells can be obtained from the American Type Culture Collection (ATCC) at 10801 University Blvd., Manassas, VA 20110-2209.

The present invention will now be illustrated by the following examples. The examples are not intended to limit the scope of the invention in any way.

Example 1: Biosensor For Detecting Streptavidin binding Peptide

A proprietary peptide which binds tightly to Streptavidin-Alkaline Phosphatase was linked to the reactive agent Sulfo-HSAB (*N*-hydroxysulfosuccimidyl-4-azidobenzoate). The Sulfo-HSAB moiety on the resulting conjugate had a photo-reactive azide group. This conjugate was added to the surface of a polypyrrole (conducting polymer) film and subjected to UV irradiation to induce covalent bonding to the film. After the ligand-functionalized film equilibrated somewhat in glass-distilled deionized water, the film was exposed to a solution of Streptavidin-Alkaline Phosphatase (SA). The result was an irreversible decrease in the resistance of the polypyrrole film (Figure 2). It may be that the binding event encourages closer packing of the individual polypyrrole chains on the surface of the polymer film. This would make it easier for current to flow from one electrode to the other through the film, and would be manifest as a decrease in electrical resistance. (With other receptor /analyte /conducting polymer systems, resistance *increases* are possible as an indication of analyte detection.).

The foregoing peptide can be obtained from Bachem Biosciences Inc. (3132 Kashiwa Street, Torrence CA 90505) as product name CS-373, lot no. B01877. The peptide has the following sequence:

H-Ala-Glu-Gly-Pro-Cys-His-Pro-Gln-Phe-Pro-Arg-Cys-Glu-Gly-Gly-Gly-Ser-Lys- Ala-$NHNH_2$ (SEQ ID No. 1).

The peptide ligand will bind to streptavidin under physiologic conditions (e.g., phosphate buffered saline, pH 7.2) with a dissociation constant (Kd) of about 200nM. The ligand has two primary amines, the alpha amine and the epsilon amine on the lysine side chain at position 18. There is also a hydrazide group at the C-terminus; this group will react with aldehydes to form a stable covalent bond.

Further evidence that such binding had occurred was afforded first by subsequent exposure of the ligand-modified polypyrrole film to *p*-nitrophenyl phosphate. The aqueous solution above the polymer-coated electrodes became yellow in color (due to the generation of *p*-nitrophenolate ion), indicating that the peptide-bound enzyme Alkaline Phosphatase was catalyzing *p*-nitrophenyl phosphate

hydrolysis. (In the absence of this enzyme, no yellow color is observed.) Second, glycerol had been added to the original SA solution to minimize SA aggregation. When the polypyrrole film was exposed to a neat glycerol solution, however, there was no further change in resistance, confirming that the original resistance change was truly due to a ligand-SA binding event. Exposing the sensor to targets for which it was not specific provided further confirmation.

The following specific methods were used to construct the sensor.

1. Synthesis of the Linker-Ligand Conjugate.

To a solution of the Dyax Inc., (Cambridge), peptide, CS-373 [4.2 mg in 300 μL of 10 mM potassium phosphate buffer, (pH 7.12)], was added 400 μL of Sulfo-HSAB, (Pierce Chemical), [2 X 10^{-6} moles of protein and 4.4 X 10^{-6} moles of Sulfo-HSAB]. The reaction proceeded at room temperature for one hour. The solution was used as is for the photo-crosslinking reaction.

2. Photochemical Covalent Bonding of the Linker-Ligand to the Conductive Polymer.

The solution of the Linker-Ligand was added directly to the surface of the cassette of conducting polypyrrole polymer. The cassette was placed on ice and irradiated in a Rayonette photolysis apparatus with a uv maximum at 254 nm for 15 minutes. The cassettes were washed with 30 mL of 10 mM potassium phosphate buffer, (pH 7.12), then with 2 X 30 mL of Deionized, Glass Distilled water, (17.0 ohm resistance). The cassettes were dried overnight then used without further treatment.

3. Binding of Strepavidin to the Linker-Ligand Modifier Cassette.

Commercially available Strepavidin from Pierce, 1 mg/mL in Phosphate Buffer was solvent exchanged with Deionized, Glass Distilled water, (17.0 ohm resistance), using a 10,000 MW cut-off Amicon Centricon. To the surface of the cassette 30 μL of the resulting strepavidin solution, (1 mg/mL), was added and the conductivity was measured.

The sensor of this Example is sometimes referred to herein as a "standard streptavidin biosensor". As discussed above, the sensor can be used to test one or a

combination of sensor components including, but not limited to, a receptor, polyfunctional linker, and semi-conductive film (with or without additives or polymer components).

Figures 19A-D show results of the sensor in the presence of various interferants. (19A) lysozyme, (19B) bovine serum albumin, (19C) pancreatin, (19D) trypsin. In each instance, the sensor showed a significant drop in resistance after adding the SA (streptavidin alkaline phosphatase) reagent.

Example 2- Proposed Biosensor for Detecting *M. tuberculosis* Bacterium

A. Synthesis of Amphiphilic Ligands and Film Formation

For the densely-packed array of ligands we have chosen to use a unique variation on the formation of Langmuir-Blodgett films, using the Schaefer modification method of film transfer. The basic concept is to construct a Langmuir-Blodgett-style film (in a trough) based on the the amphiphilicity of the base linkers at the air-water interface (see Figure 18). The Langmuir-Blodgett film will actually be a monolayer of amphiphilic derivatized ligands that self-assembles using the polar head group of the amphiphile to orient the hydrophilic end of the molecule in the aqueous subphase while the hydrophobic portion of the molecule will remain above the interface. This is the concept of floating a drop of oil on the surface of water as originally described by Benjamin Franklin when he used a layer of oil to induce a calming influence over the water in the Clapham ponds2 . In this process water will be the subphase and the amphiphile will be a 1 -maleimidyl- 1 8-[p-azophenyl] octadecane . This bifunctional reactive am phi phile [as well as other members of its family], while not commercially available, is readily synthesized from the Friedel-Crafts alkylation of 1-[N-maleimidyl]-18-chlorooctadecane and azobenzene with AICI3. The synthesis is shown in Figure 4.

The trough will be based on the design of Lin et al.3 and will not be discussed further. Once the amphiphile is placed on the surface the trough will compress the two dimensional surface to a point where the area per molecule is approximately 22 A^2. This occurs when the surface pressure is measured to be greater than approximately

20 dyn/cm. Under these conditions the surface is referred to as a "solid phase" or liquid condensed stated. This defines the Langmuir-Blodgett film and is shown in Figure 5. The Langmuir-Blodgett film now has the polar head groups in the aqueous subphase and the alkyl tails densely-packed and held in position by hydrophobic interactions. It should be noted that the bifunctional nature of the amphiphile has not been altered. The polar head group is susceptible to nucieophilic attack and the azo group on the hydrophobic tail is photoactive.

B. Formation and Transfer of the Langmuir-Blodgett linker film

The next step in this scheme is to covalently bond the thick "lawn" of linkers to the conducting polymer. To effect the Langmuir-Blodgett bioconjugate film transfer to the conducting polymer substrate, we propose to use the Schaefer method. In the Schaefer method, the substrate (with polypyrrole already deposited onto it, for example) is placed on top of the Langmuir-Blodgett film. A film of this type (i.e., based on stearic acid) has been accurately measured to extend 25 A above the surface of the subphase. Therefore, placement of the substrate to the contact point of the surface of the Langmuir-Blodgett film may be accomplished with great facility. Under the standard Schaefer method, the film is then lifted free of the subphase, thereby forming a thin layer on the substrates. One of the obvious difficulties with this methodology is freeing the film from the subphase without disruption due to gravity, surface tension and van der Weals interactions. To circumvent this issue we propose the following to ensure that the film has the structural integrity to survive transfer. Upon irradiation with a UV lamp, the azo moiety on the hydrophobic tail will form a nitrene group, which will insert into carbon-hydrogen bonds of the polypyrrole film, affording a covalent bond between the linker film and the conductive film When the substrate is then lifted from the water's surface, the covalently-bonded, densely packed linker film will remain attached to the substrate. The linker film will provide the high concentration of binding sites for subsequent attachment of biospecific ligands needed for signal amplification. This sequence is depicted in Figure 6.

C. Attachment of the Bioligand to the Linker

With the conducting polymer and densely-packed linker films stitched together, the next step is to covalently bond the ligand to the linker. To capture the

mycobacterium we propose to use a commercially-available antibody specific for the agent. The antibody, which is an IgG molecule found in the immune system, may be rendered chemically-active by reducing the disulfide bonds connecting the two halves of the IgG. These fragments of IgG have been commercially exploited by Pierce Chemical and others due to their extremely tight binding constants, approaching those of the "irreversible" biotin-streptavidin conjugate, Ka= $10^{15}M^{-1}$,[7] (Ka = association constant). This is the basic principle used in antibody affinity chromatography. The IgG may be reduced with mercaptoethylamine to the corresponding sulfhydryl in situ the free sulfhydryl of the antibody fragment will then add in a nucleophilic sense to the maleimidyl terminus of the Langmuir-Blodgett linker film. This concept is demonstrated in Figure 7.

D. Testing of the Biosensor

The sensor, thus constructed, will bind tightly and specifically to Mycobacterium tuberculosis. Given that the mycobacterium is two orders of magnitude larger than any one single ligand, it is likely that several ligands will bind to it at once. The binding events v fill mechanically perturb the dense self-assembled linker nanostructure. Since the linker "lawn" is covalently-bonded to the surface of the conducting polymer (e.g., polypyrrole), the conductive film will also be perturbed, and its conductivity will be altered. In this way, the direct binding of the mycobacterium to the ligand will be converted to an electrical signal via the underlying conductive polymer. The signal derived from the presence of a single mycobacterium will be significantly enhanced due to the binding of the pathogen with many tens of ligands, each of which will contribute to the electrical resistance change. The sensor will be exposed to the M. tuberculosis in aqueous form, and the conductivity measured as a function of analyte concentration. Interferants will include non specific bacteria as well as other biological fluids such as blood, urine and sputum. Titration of the mycobacterium will be done to determine the sensitivity of the biosensor.

With this technology, it will be possible to construct an array of biosensors that will individually alarm for each of the causative agents for food poisoning. The array responses could be used to identify each species of potentially pathogenic

organism present and quantitatively assess the potential risk from contamination. It is thus feasible to construct a complete sensor that would include the sensor array, all the electronics, the display, and a fully-developed logic.

Example 3- Proposed Biosensor For Detecting Pediatric *S. Pneumoniae* and *Varicella*

A. Synthesis of rod-coil structures with electophilic coil termini

The basic synthesis of the rod-coil macromolecule will be accomplished using the techniques pioneered by Stupp and co-workers . The rod-coil assembly strategy is to independently synthesize the rod-like portion of the macromolecule, compound 3 in Figure 9, by a condensation reaction between two stiff units, compounds 1 and 2. The length of the rod like molecule may be controlled to some degree by the choice of starting materials. However, it is critically important that this rigid segment not exceed a certain maximum length, or else it will assume a helical conformation and cease to be purely linear. Should this segment become non-linear, its ability to "pack" in an orderly array. For the synthesis of the coil-like segment, a polyisoprene unit is synthesized using anionic polymerizations. The molecule that initiates the anionic polymerization will be a derivatized isoprene fitted with an electrophilic maleimide for later covalent attachment of the antibody fragment (Figure 9, compound 4). The final condensation of the rod-like segment with the polymer coil leads to the rod-coil structure, compound 5. The molecular weight of these macromolecules is reported to be between 8 and 10 kDa, as determined by gel permeation chromatography against polystyrene molecular weight standards. The polydispersity of these molecules ranges from 1.03 to 1.13, and the volume fraction for the rod segment is approximately 0.2. NMR as well as TOF mass spectrometry in addition to molecular weight determination will be used to characterize these macromolecules.

B. Formation of the amphiphilic nanostructures and intercalation into the conducting polymer

In order to facilitate the self-assembly of the rod-coils into the mushroom-like structures, thin films of the macromolecules are cast from dilute solutions of cyclohexane onto carbon coated glass slides. The rod-like portion of the rod-coil is

soluble in the organic medium. After the solutions have stood unstirred for 24 hours, the solvent is removed under vacuum. The resulting ultra-thin films of the self-assembled mushroom-like nanostructures are floated off of the glass slide using an aqueous solution of the conducting polymer. The aqueous solution at this point consists of the conducting polymer solubilized in the aqueous phase, and the self-assembled rod-like segments intercalated into that phase. The coil-like portions of the rod-coil structures remain above the air/water interface. The aqueous medium is cast onto bare gold-on-glass slide substrates, dried, and annealed. Characterization of this surface following the annealing step will be accomplished using atomic force microscopy, as well with transmission electron microscopy7. In practice, we expect to find a densely packed array of "mushroom-caps", with the aggregated self-assembled coil segments on the surface of the conducting polymer, and the aggregated self-assembled rod segments intercalated into the conducting polymer matrix. The overall features of these nanostructures are shown in Figure 10.

C. Covalent bonding of the antibody fragment to the electrophilic coil terminus

With the formation of the basic sensor platform completed, the next step is to covalently bond the bioligand to the surface of the "mushroom" nanostructure. The strategy is to attach an antibody fragment specific to either of the target biomolecules, S. pneumoniae or Varicella. Antibodies for these causative agents will be fragmented in situ using standard techniques8, leaving an active sulfhydryl function on the antibody fragment. These active fragments will add directly to the maleimide moieties at the termini of the mushroom coil segments. To demonstrate that the technique will work, fluorescent microscopy will be used to quantify the number of fluorescent antibody probe fragments present. The ability of the antibody to bind to the cells and viral particles of interest will be demonstrated by using specific sandwich assays, similar to the motif of an Enzyme-Linked Immunosorbent Assay (ELISA). In the ELISA technique, the cells or viral particles will be bound to the conjugated antibody fragments and secondary antibodies will be incorporated that will have specific reporter functions. The readout may be fluoresence, radioactivity, or an enzyme assay. This will allow us to quantify the number of binding events possible on the surface of the biosensor. The covalent bonding is shown in Figure 11.

D. Testing of the real-time pediatric biosensor

The final test of the biosensor will be to expose the surface of the conducting polymer/intercalated rod-coil/bioligand to the target organism. The system will be a success if the binding event causes a change in conductivity. As negative controls, the system will be challenged with a variety of biofluids and/or simulants, as well as with organisms not specific for the antibody in use. Titration of the biosensor with the target bacterium and viral particle will determine the sensitivity of the instrument. It is expected that having multiple "mushroom"/film attachment sites and multiple antibody/bacterium binding events will result in an amplified detection signal.

Example 4- Proposed Biosensor for Detecting Food Poisoning Pathogens

A. Synthesis of Dendrimers with Bifunctional Surfaces

Most syntheses of dendrimers evolve from the repetitious alternation of growth reactions and activation reactions. The scope and details of these reactions have been reviewed elsewhere'. There are four methodologies that are incorporated into the synthesis of dendrimers, namely: divergent growth, convergent growth, growth using hypercores with branched monomers, and double exponential and mixed growth. It is this latter protocol that we will utilize to develop the bifunctional surfaces. During the growth phase, and generally by the fourth generation, the so-called "starburst limit" has been reached and the surface is so crowded that no further growth chemistry can occur. The basic chemistry generally involves simple addition/substitution reactions, as shown in Figure 13. These units will then be the core elements for the double exponential mixed growth protocol. The general scheme for the growth of the first surface [A] is shown in Figure 14. This nanostructure is a dendron to which new branching moieties may be added to generate the second surface, [B]. This is demonstrated in Figure 15. In our scheme, surface A will contain a hydrophobic arm terminating in a photoactive azobenzene group. Surface B will contain a relatively-polar electrophilic maleimide. Surface A will ultimately bond covalently to the conducting polymer, while surface B will be covalently bonded to the bioligand specific for the target bacteria.

B. Self Assembly and Attachment of the Dendrimer to the Surface of the Conducting Polymer

Due to the bipolar nature of their surfaces, the dendrimers will have the tendency to orient themselves based on the polarity of the processing solvents used. We propose to disperse the dendrimer in an aqueous medium. A thin dendrimer film will form at the air-water interface, with the hydrophobic surface oriented out of the water, and the hydrophilic surface facing down into the water. Once the film has been formed, the conducting polymer-coated substrate will be turned upside-down, with its surface parallel to that of the water, and lowered until it just comes into contact with the dendrimer film at the water's surface. The system will then be irradiated with UV light. As a result, the azobenzene-functionalized hydrophobic termini of the dendrimer will covalently bond to the surface of the conducting polymer, immobilizing the dendrimer. When the substrate is then raised from the water, the hydrophilic dendrimer's surface B. which had been oriented into the aqueous subphase, will be expressed on the outermost film surface, and will be available to participate in subsequent ligand-attachment chemistry (Figure 16). Similar approaches have been previously reported.

C. Covalent Bonding of the Bioligand to Surface B of the Dendrimer

With the dendrimer now immobilized on the surface of the conducting polymer, the next step is the introduction of the bioligand. Each bioligand we have chosen is a fragment of the IgG antibody that is specific to one of the five pathogens of interest mentioned above. These fragments of IgG have been commercially exploited by Pierce Chemical and others due to their extremely tight binding, with binding costants approaching that of the "irreversible" biotin-streptavidin conjugate, $K_a = 10^{15} M^{-1}$, (Ka= association constant).

Binding of this kind is widely used in antibody affinity chromatography. The antibodies used here may be rendered chemically-active by reducing the disulfide bonds connecting the two halves of the IgG. Specifically, the IgG may be reduced with mercaptoethylamine to the corresponding sulfhydryl in situ 5, and the free sulfhydryl of the antibody fragment will then add in a nucleophilic sense to the

maleimidyl terminus of the B surface of the dendrimer. This concept is demonstrated in Figure 17.

D. Testing of the Biosensor

With the dense coating of the bioactive dendrimer on the surface of the conducting polymer, exposure of the conductive film to the target organism should lead to a binding event that will significantly perturb the film's surface, resulting in a measureable conductivity change. The system will be challenged with a variety of biofluids, as well as with irrelevant organisms (targets for which the dendrimer-bound ligands are not specific) as negative controls. Titration of the biosensor with the target organism will be used to determine the sensitivity of the sensor. It is expected that having multiple dendrimer/ film attachment sites and multiple antibody/ bacterium binding events will result in an amplified detection signal.

With this technology, it will be possible to construct an array of biosensors that will individually alarm for each of the causative agents for foodborne illness. The array responses could be used to identify each species of potentially pathogenic organism present and quantitatively assess the potential risk from contamination. It is also feasible to construct a complete sensor that would include the sensor array, all the electronics, the display, and a fully-developed logic. The sensor will be able to accurately and selectively identify and quantify the pathogens causing foodborne illnesses in real time.

All references disclosed herein are incorporated by reference including the following specific references:

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What is claimed is:

1. An implementation for detecting at least one analyte in a sample, the implementation comprising:

a. a sensor array comprising one or a plurality of test sensors in which each test sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iii) a polyfunctional linker comprising a first end attached to a receptor and a second end,

wherein, the semi-conductive film comprises at least one polymer material attached to the second end of the polyfunctional linker, the film being conducive to registering analyte binding as a change in at least one electrical property of the film, wherein the change is indicative of the presence of the analyte in the sample.

2. The implementation of claim 1, wherein the implementation further comprises a detector operably linked to at least one of the sensors in the array, the detector being adapted to output any change in the electrical property to a user of the implementation.

3. The implementation of claim 2, wherein the detector is an ohm or capacitance vector.

4. The implementation of claim 1, wherein the sensor array comprises a plurality of interdigitated electrodes.

5.The implementation of claim 4, wherein the interdigitated electrodes are each connected to the detector.

6.The implementation of claim 2, wherein the detector is operably linked to a power supply.

7.The implementation of claim 1, wherein the semi-conductive film comprises at least one layer of polymer material.

8. The implementation of claim 1, wherein the semi-conductive film provides an increase or decrease in electrical conductivity between the electrodes in the presence of the analyte.

9.The implementation of claim 1, wherein the semi-conductive film provides an increase or decrease in capacitance or impedance between the electrodes in the presence of the analyte.

10.The implementation of claim 1, wherein each sensor in the array comprises a plurality of polyfunctional linkers having a density of between from about $3x10^{11}$ to about $8x10^{12}$ as determined by atomic force microscopy.

11.The implementation of claim 10, wherein the plurality of polyfunctional linkers are configured as an aggregate.

12.The implementation of claim 11, wherein each aggregate has a length of between from about 5 nm to about 100 nm.

13. The implementation of claim 1, wherein the polyfunctional linker is bifunctional.

14. The implementation of claim 13, wherein the bifunctional linker comprises a rod-like component.

15.The implementation of claim 14, wherein the rod-like component of the bifunctional linker has a height of between from about 0.5 nm to about 50 nm.

16.The implementation of claim 13, wherein the bifunctional linker has a molecular weight of between from about 1 kDa to about 20kDa as determined by gel permeation chromatography.

17.The implementation of claim 14, wherein the rod-like component has a volume of between from about 60 nm^3 to about 150 nm^3 as determined by light scattering.

18.The implementation of claim 14, wherein the rod-like component has a radius of gyration of between from about 1 nm to about 100 nm as determined by light scattering.

19.The implementation of claim 13, wherein the bifunctional linker comprises, prior to reaction with the receptor and the semi-conductive film, an electrophilic group on the first end and a photoactivatable group on the second end.

20. The implementation of claim 19, wherein the receptor is covalently attached to the electrophilic group of the bifunctional linker.

21. The implementation of claim 19, wherein the polymeric material is covalently linked to the photoactivatable group of the bifunctional linker.

22.The implementation of claim 19, wherein the electrophilic group is polar and the photoactivatable group is hydrophobic.

23.The implementation of claim 13, wherein the bifunctional linker comprises, prior to reaction with the receptor and semi-conductive film, a photoactivatable group on the first end and an electrophilic group on the second end.

24.The implementation of claim 21, wherein linker is attached to the backbone of the polymeric material.

25.The implementation of claim 19, wherein the photoactivatable group is an optionally substituted azobenzene group.

26.The implementation of claim 19, wherein the electrophilic group is an optionally substituted maleimide group.

27.The implementation of claim 13, wherein the bifunctional linker is from about 0.5 nm to about 50 nm in length.

28.The implementation of claim 13, wherein the bifunctional linker is water soluble.

29. The implementation of claim 19, wherein the bifunctional linker is represented by the following formula:

A-B-C

wherein,

A is defined as the an optionally substituted maleimide group,

B is defined as a spacer having a length of from between about 1 Angstrom to about 50 Angstroms; and

C is defined as an optionally substituted azobenzene group.

30. The implementation of claim 29, wherein B is further defined as a C_1 to C_{50} alkyl group, alkyl polyol, polyoxyalkyl group, polymethylol, or polyoxyethylene group.

31. The implementation of claim 1, wherein the polyfunctional linker and the receptor is represented by the following formula:

wherein P is defined as a dendrimer, X represents an integer of 1 or greater, each M represents the receptor, y represents an integer of 1 or greater; and

* indicates that the receptor is associated with the dendrimer.

32.The implementation of claim 31, wherein the dendrimer has a diameter of from between about 100 Anstroms to about 1000 Angstroms.

33.The implementation of claim 31, wherein the dendrimer, prior to reaction with the receptor and the semi-conductive film, has at least one electrophilic group on a first end and at least one photoactivatable group on a second end of the dendrimer.

34. The implementation of claim 33, wherein the receptor is non-covalently attached to the electrophilic group on the first end of the dendrimer.

35.The implementation of claim 33, wherein the photoactivatable second end of the dendrimer is further bound to the polymer material.

36.The implementation of claim 35, wherein the dendrimer is further bound to the backbone of the polymer material.

37.The implementation of claim 33, wherein the electrophilic group is polar and the photoactivatable group is hydrophilic.

38. The implementation of claim 1, wherein the polyfunctional linker is intercalated within the polymeric material.

39.The implementation of claim 1, wherein the polymeric material comprises a plurality of conjugated carbon atom bonds.

40.The implementation of claim 1, wherein the polymer material further comprises at least one chemical group adapted to join the second end of the polyfunctional linker to the polymer material.

41.The implementation of claim 1, wherein the polymeric material has an electrical conductivity between from about 10^{-16} to about 10^{-3} $ohm^{-1}.cm^{-1}$.

42.The implementation of claim 1, wherein the polymeric material has a thermal stability of up to about 200°C as determined by thermal gravimetric analysis.

43.The implementation of claim 1, wherein the polymeric material comprises a conductive epoxy resin.

44.The implementation of claim 1, wherein the polymeric material comprises a polymer, co-polymer, graft co-polymer, or polymer alloy.

45.The implementation of claim 39, wherein the polymeric material is an optionally substituted polyaniline, polythiophene, polypyrrole, polyparaphenylene, or polyparaphenylene vinglene.

46.The implementation of claim 1, wherein the semi-conductive film further comprises at least one additive or a component for increasing or decreasing electrical conductivity.

47.The implementation of claim 46, wherein the additive is an optionally substituted lower alkyl diamine.

48.The implementation of claim 46, wherein the component is at least one of a metal, metallic alloy, or a carbon.

51.The implementation of claim 48, wherein the metal is gold, silver, copper, platinum, or nickel.

52.The implementation of claim 48, wherein the carbon material is carbon black, graphite or carbon nanotubes.

53. The implementation of claim 47, wherein the lower alkyl diamine is hexanediamine.

54.The implementation of claim 1, wherein the receptor is capable of binding at least one analyte.

55.The implementation of claim 1, wherein the receptor is a nucleic acid or a derivative thereof.

56. The implementation of claim 1, wherein the receptor is a peptide, polypeptide, protein, lipid or a glycolipid.

57.The implementation of claim 56, wherein the protein is a surface modified enzyme; or a substrate binding fragment thereof.

58.The implementation of claim 56, wherein the protein is an antibody; or an antigen binding fragment thereof.

59.The implementation of claim 55, wherein the nucleic acid is RNA or a derivative thereof.

60.The implementation of claim 59, wherein the nucleic acid is catalytic DNA or RNA.

61.The implementation of claim 54, wherein the analyte is one of a nucleic acid, peptide, polypeptide, protein, enzyme substrate, lipid, fungus, glycolipid, antigen, virus, prion, metazoan cell; or receptor binding fragment thereof.

62. The implementation of claim 61, wherein the analyte is a bacterial or protozoan cell.

63.The implementation of claim 1, wherein the sample comprising the analyte is a liquid, gas, vapor, mist or an emulsion.

64.The implementation of claim 63, wherein the liquid is a flow stream.

65. The implementation of claim 64, wherein the implementation further comprises a pump operably linked to the implementation.

66. The implementation of claim 1, wherein the implementation further comprises a control sensor, the control sensor comprising essentially the same components of the test sensor with the proviso that the control sensor not include the receptor.

67. The implementation of claim 2, wherein the implementation further comprises a computational system operably linked to the detector for manipulating the output.

68. The implementation of claim 67, wherein the output is stored by the computational system and optionally processed prior to display to the user.

69. The implementation of claim 67, wherein the manipulation comprises comparing the output to a standard analyte concentration curve and determining the amount of analyte in the sample.

70. The implementation of claim 2, wherein the output is displayed to the user essentially in real-time.

71.The implementation of claim 1, wherein the implementation is capable of remote detection of an analyte.

72. The implementation of claim 1, wherein the implementation further comprises a wireless communication system.

73. An implementation for detecting an analyte in a sample, the implementation comprising:

a. a sensor array comprising one or a plurality of sensors in which each sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber having dimensions less than about 10000 nm x 10000 nm,

ii) a semi-conductive film having a thickness of between from about 50 Angstroms to about 1000 Angstroms and positioned at least in the chamber, the film providing electrical contact between the electrodes; and either

iii) a bifunctional linker having a length of from between about 2 nm to 10 nm and comprising a first end attached to a protein, nucleic acid; or analyte binding fragment thereof, the linker further comprising a second end, or

iv) a dendrimer having a diameter of from between about 2 Angstroms to about 100 Angstroms and comprising a first end attached to the protein, nucleic acid; or analyte binding fragment, the dendrimer further comprising a second end,

wherein, the semi-conductive film comprises at least one of an optionally substituted polyaniline, polyparaphenylene, polyparaphenylene vinylene, or polythiophene polymer in which the second end of the bifunctional linker or dendrimer is attached to the polymer, the film being capable of registering analyte binding to the protein, nucleic acid; or fragment thereof as a change in electrical conductivity of the film, wherein the change is indicative of the presence of the analyte in the sample.

74. A method for detecting an analyte in a sample, the method comprising contacting the sample with an implementation for detecting the analyte, the implementation comprising:

a. a sensor array comprising one or a plurality of test sensors in which each test sensor comprises:

i) a set of electrodes configured with an insulating surface to form a chamber,

ii) a semi-conductive film positioned at least in the chamber, the film being in electrical contact with the electrodes; and

iv) a polyfunctional linker comprising a first end attached to a receptor and a second end,

wherein, the semi-conductive film comprises at least one polymer material attached to the second end of the polyfunctional linker, the film being conducive to registering analyte binding as a change in at least one electrical property of the film, wherein the change is indicative of the presence of the analyte in the sample.

75. A method for making the implementation of claim 1, the method comprising the steps of:

a. layering the semi-conductor film within the chamber,

b. contacting a polyfunctional linker with the film under conditions sufficient to bind the linker to the film surface or within the film; and

c. contacting the receptor to the polyfunctional linker under conditions sufficient to bind the receptor to the polyfunctional linker to make the implementation.

76. A method for making the implementation of claim 1, the method comprising the steps of:

a. layering the semi-conductor film within the chamber,

b. contacting a polyfunctional linker with a receptor under conditions sufficient to form a linker-receptor binding complex; and

c. contacting the linker-receptor binding complex to the film under conditions that bind the polyfunctional linker end of the linker-receptor binding complex to the film surface or within the film to make the implementation.

77.The method of claim 75 or 76, wherein the method further comprises repeating step a) at least once to form a multi-layer film having a thickness of from between about 50 Angstoms to about 1000 Angstroms.

78. The method of claim 75 or 76, wherein the polyfunctional linker is configured as a thin film prior to or during binding to the semi-conductor film.

79.The method of claim 75 or 76, wherein the method further comprises making the thin film using a Langmuir-Blodgett device and transferring the thin film from the device to the insulating surface of the implementation.

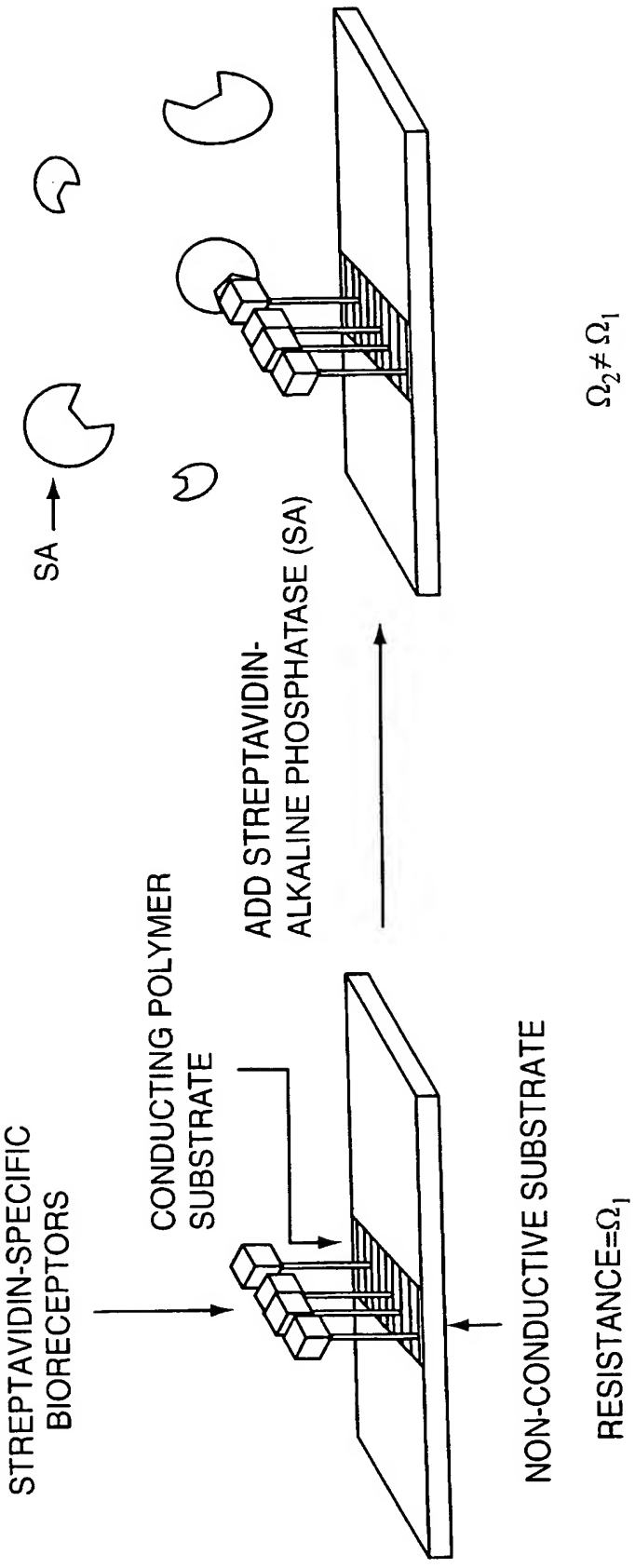

FIG. 1

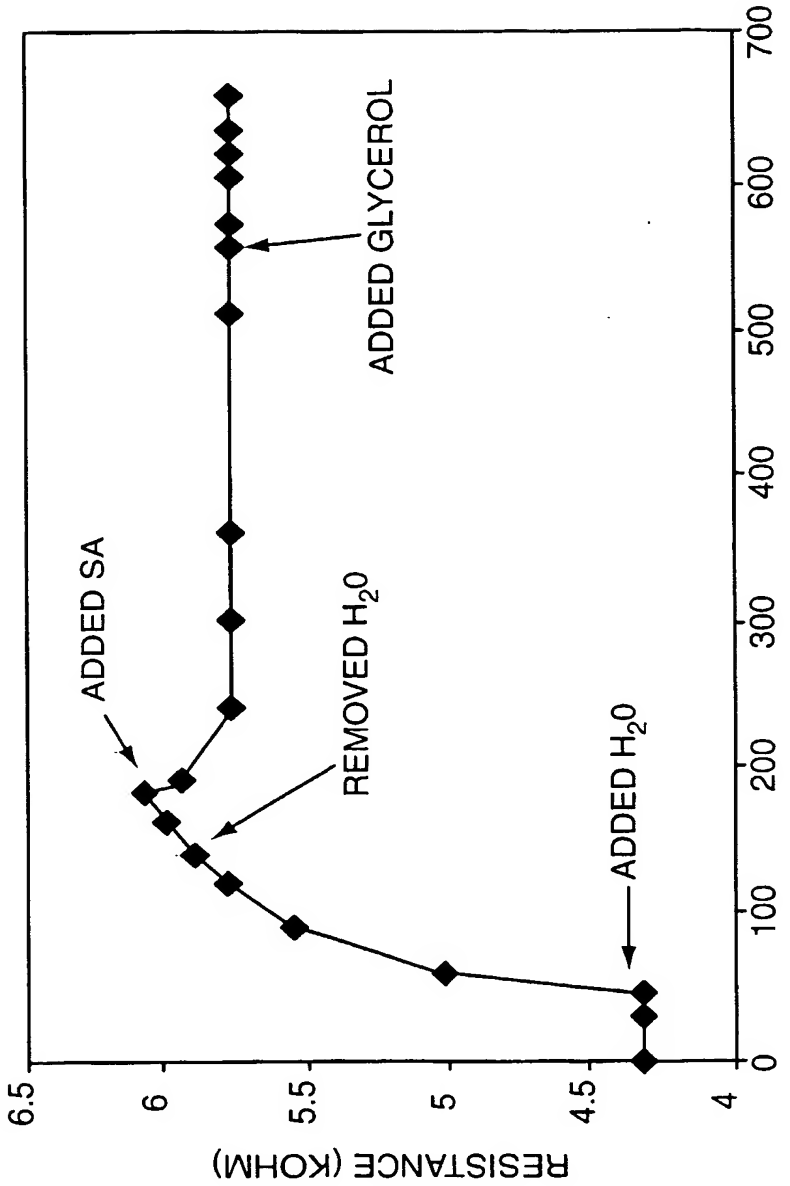

FIG. 2

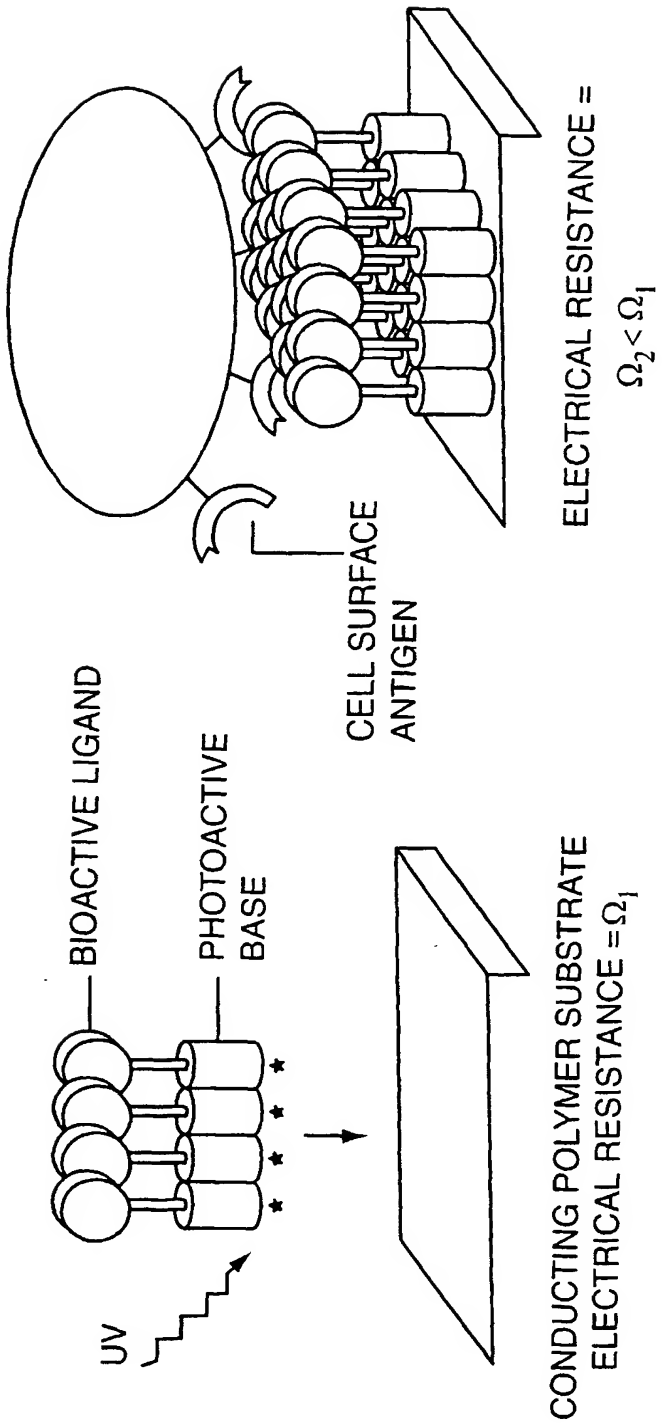

FIG. 3

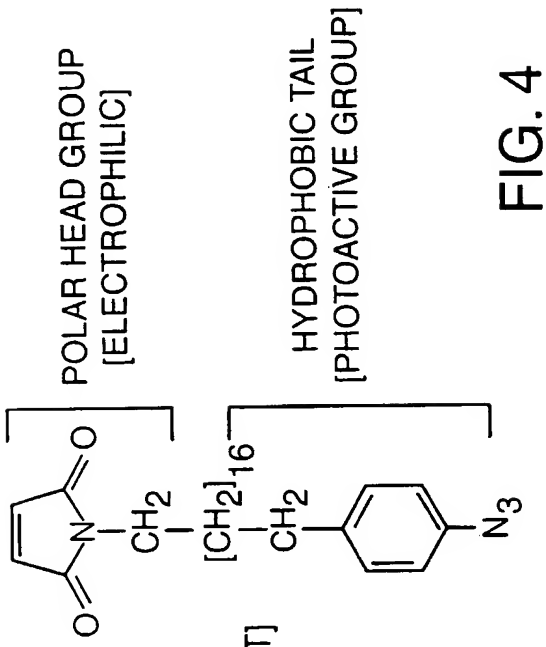

FIG. 4

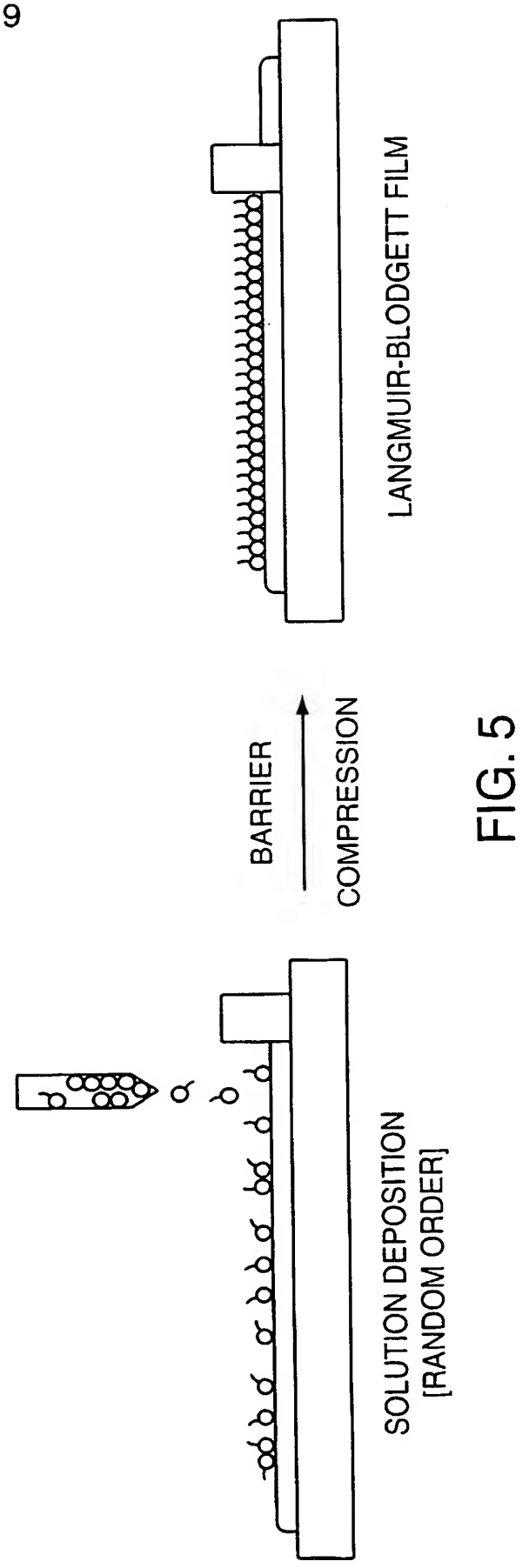

FIG. 5

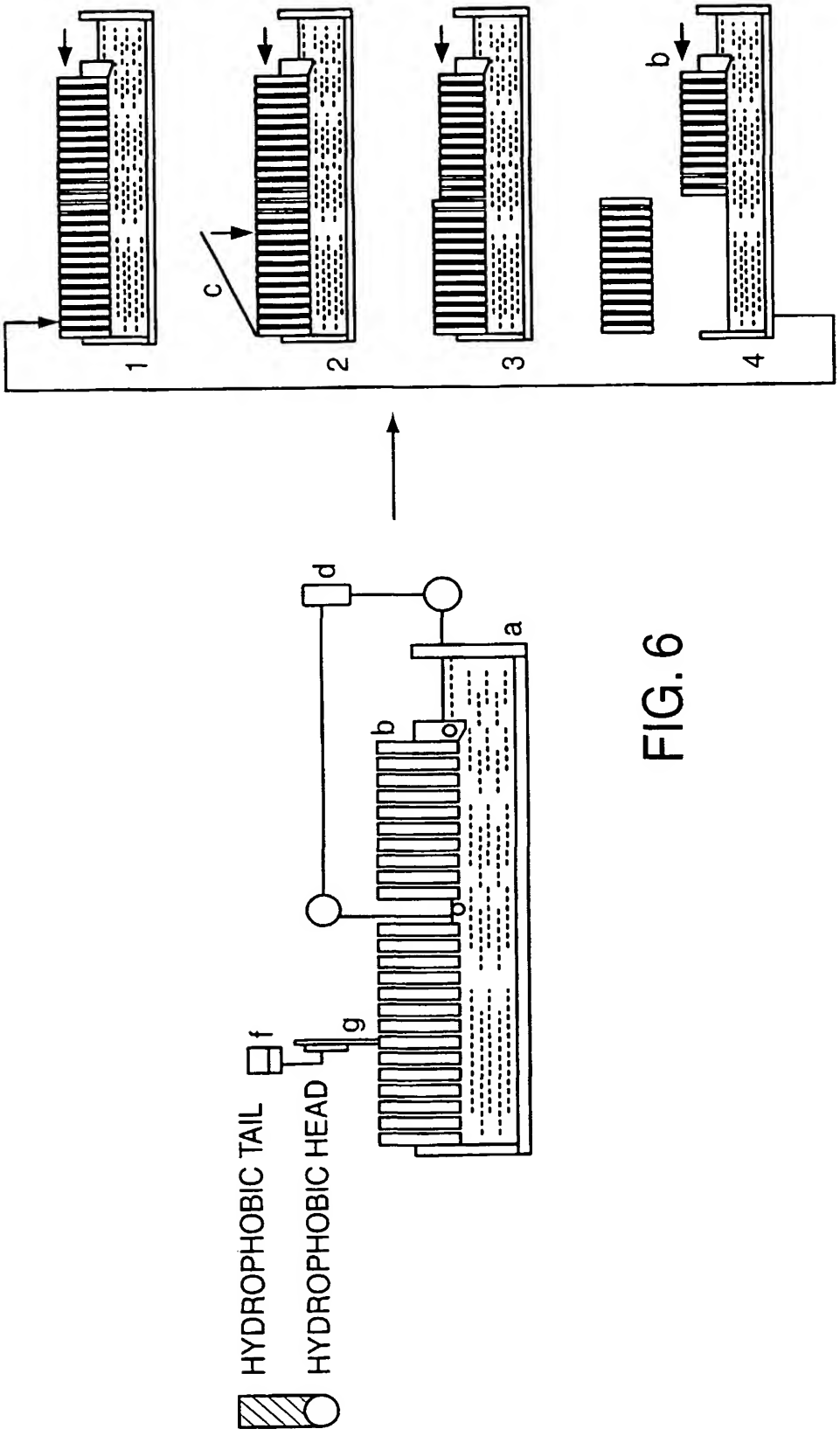

FIG. 6

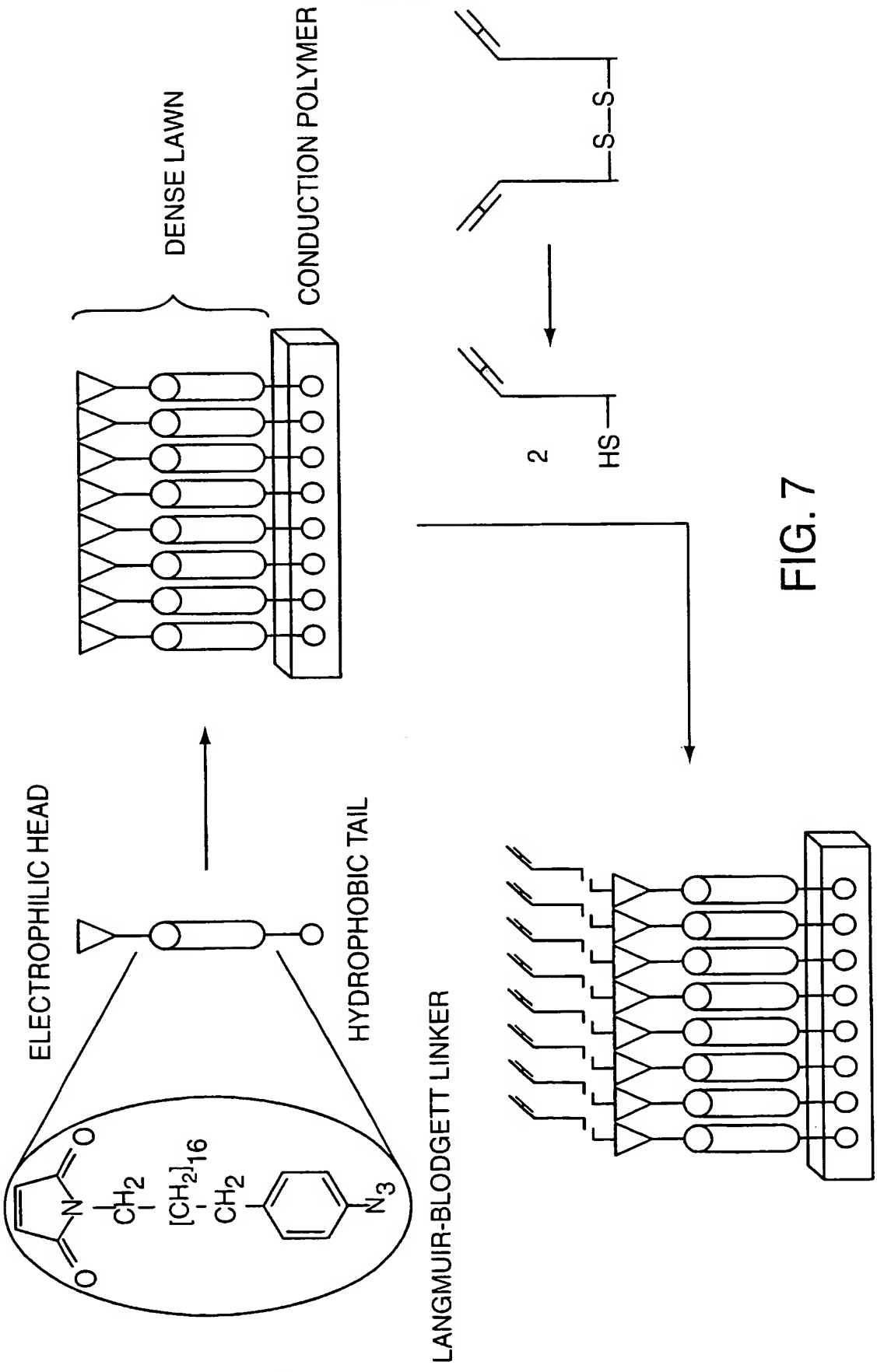

FIG. 7

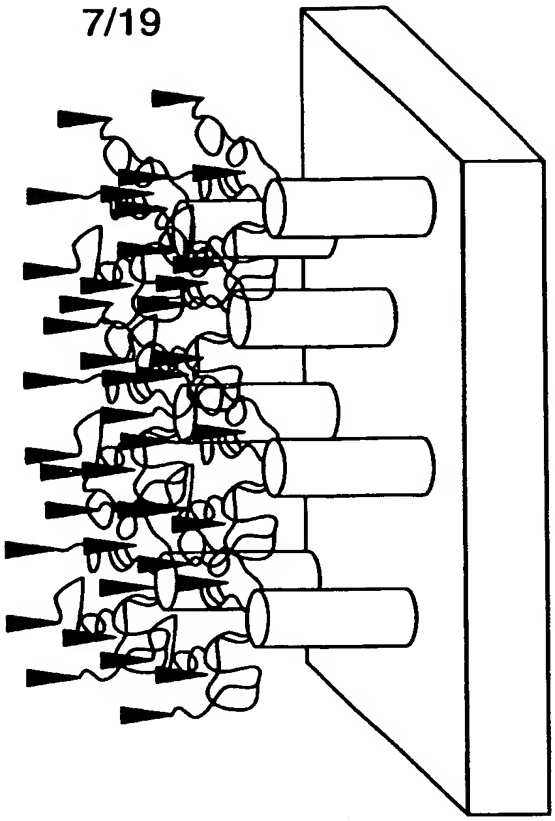

FIG. 8B

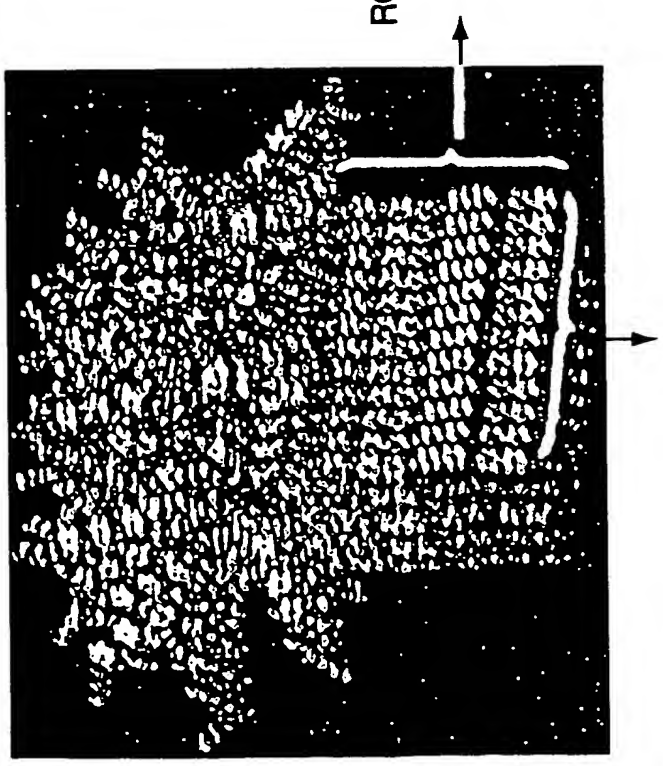

FIG. 8A

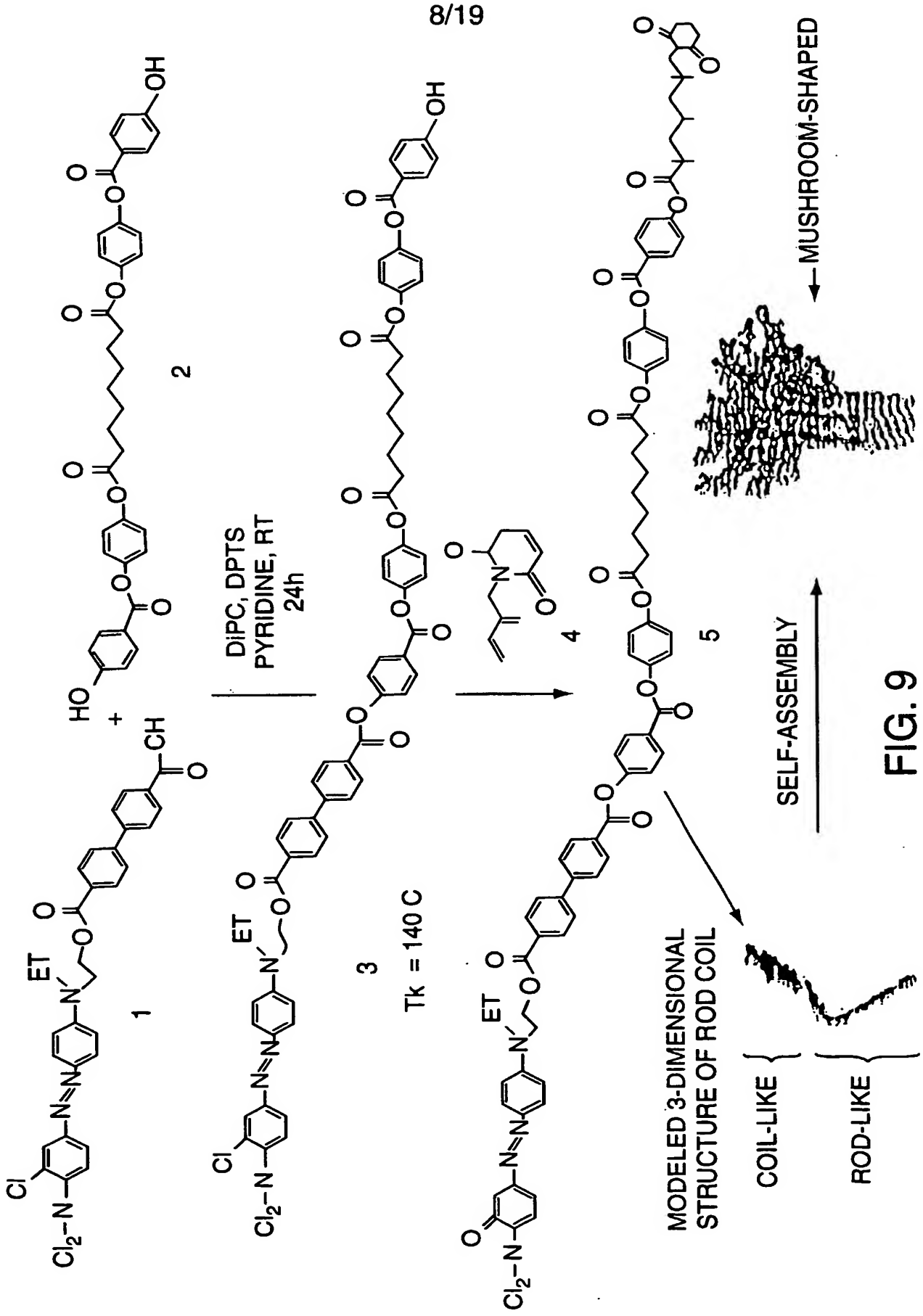

FIG. 9

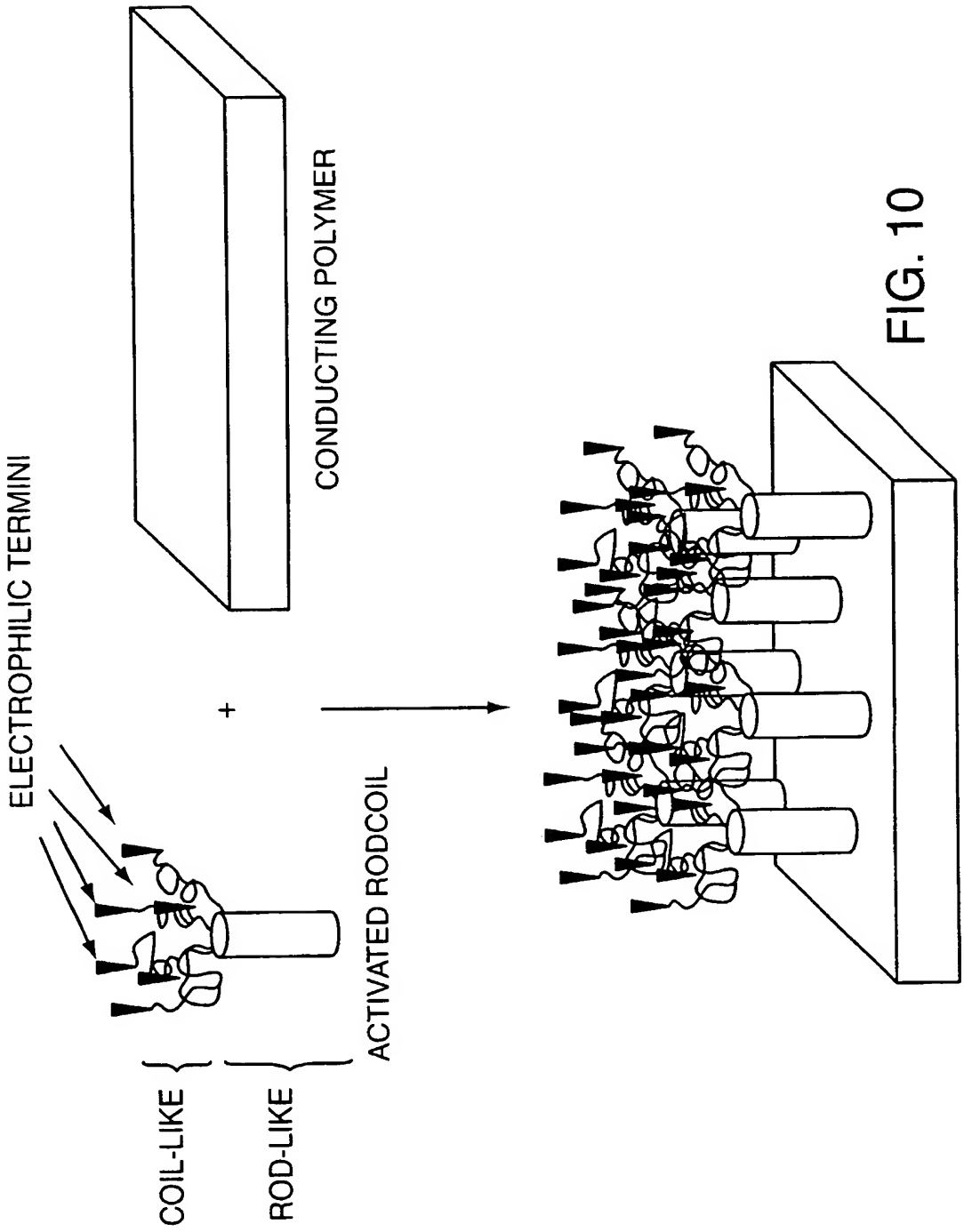

FIG. 10

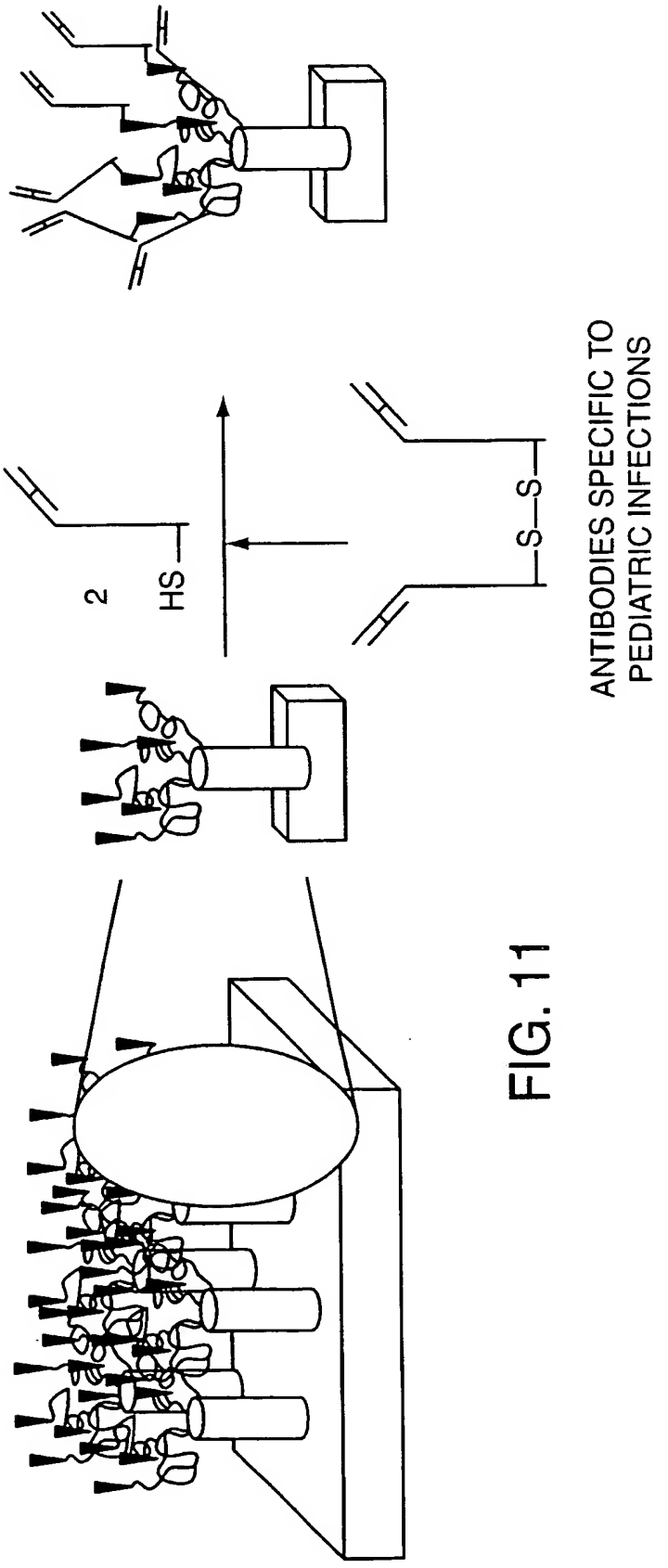

FIG. 11

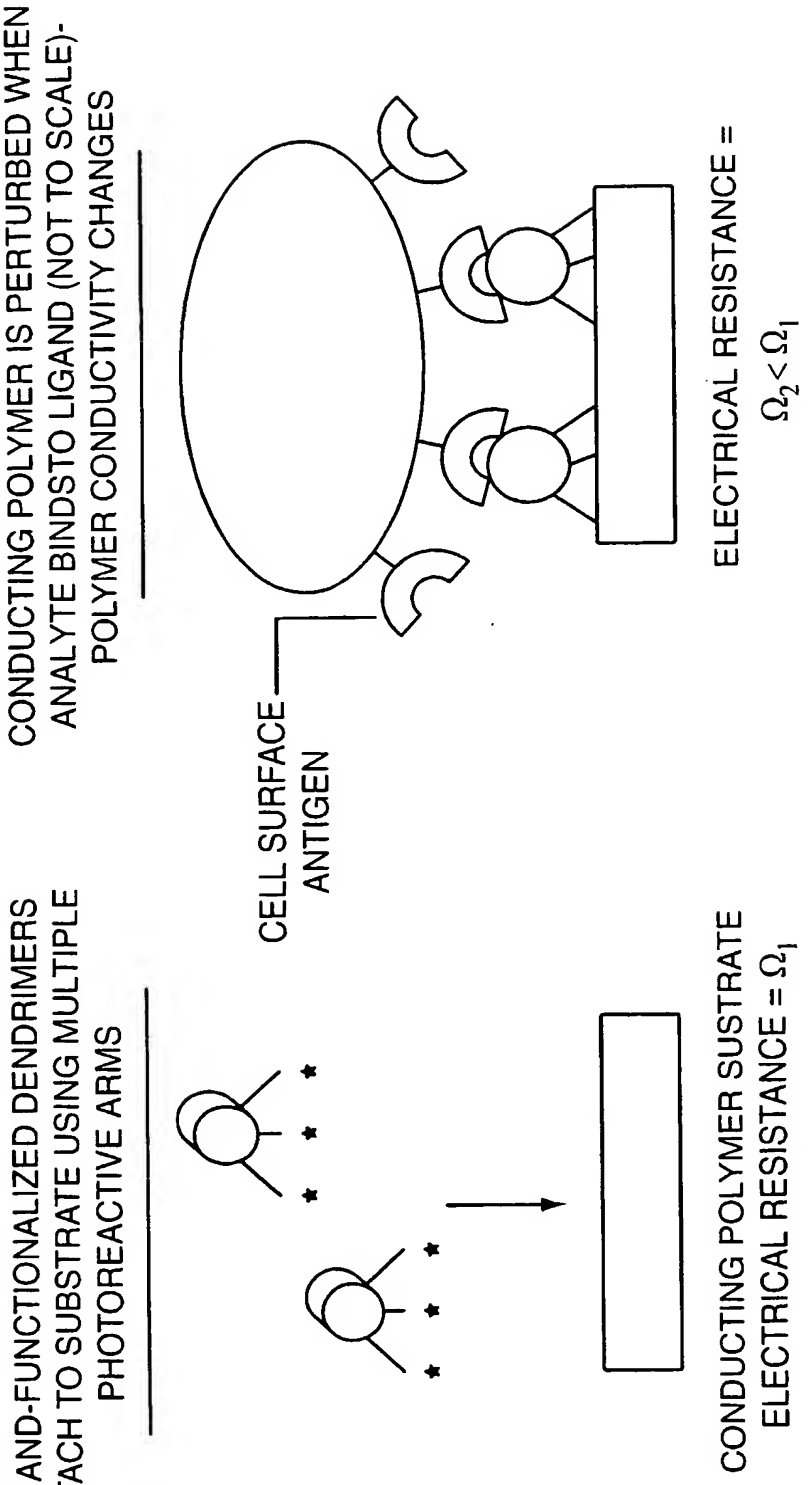

FIG. 12

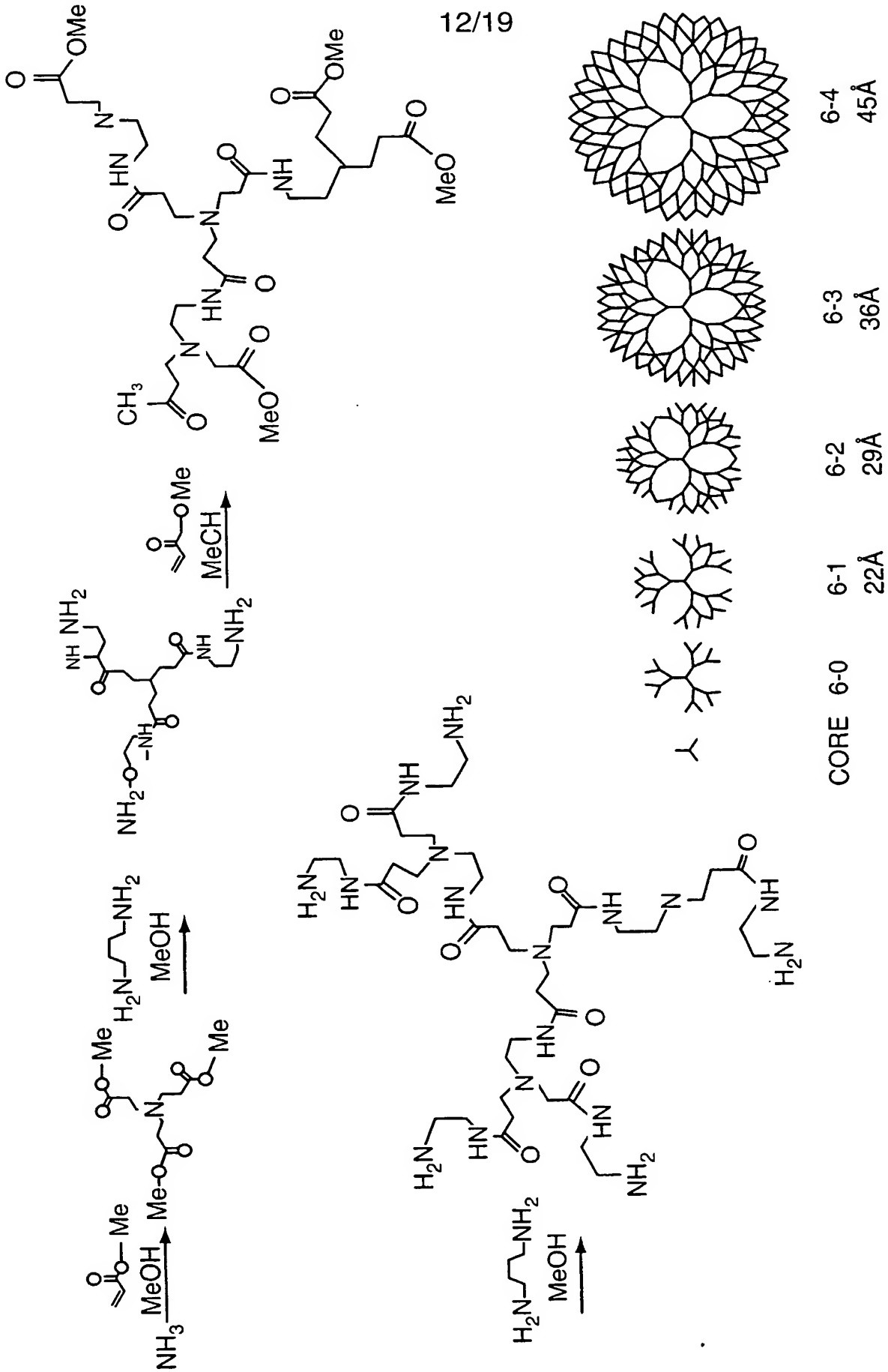

FIG. 13

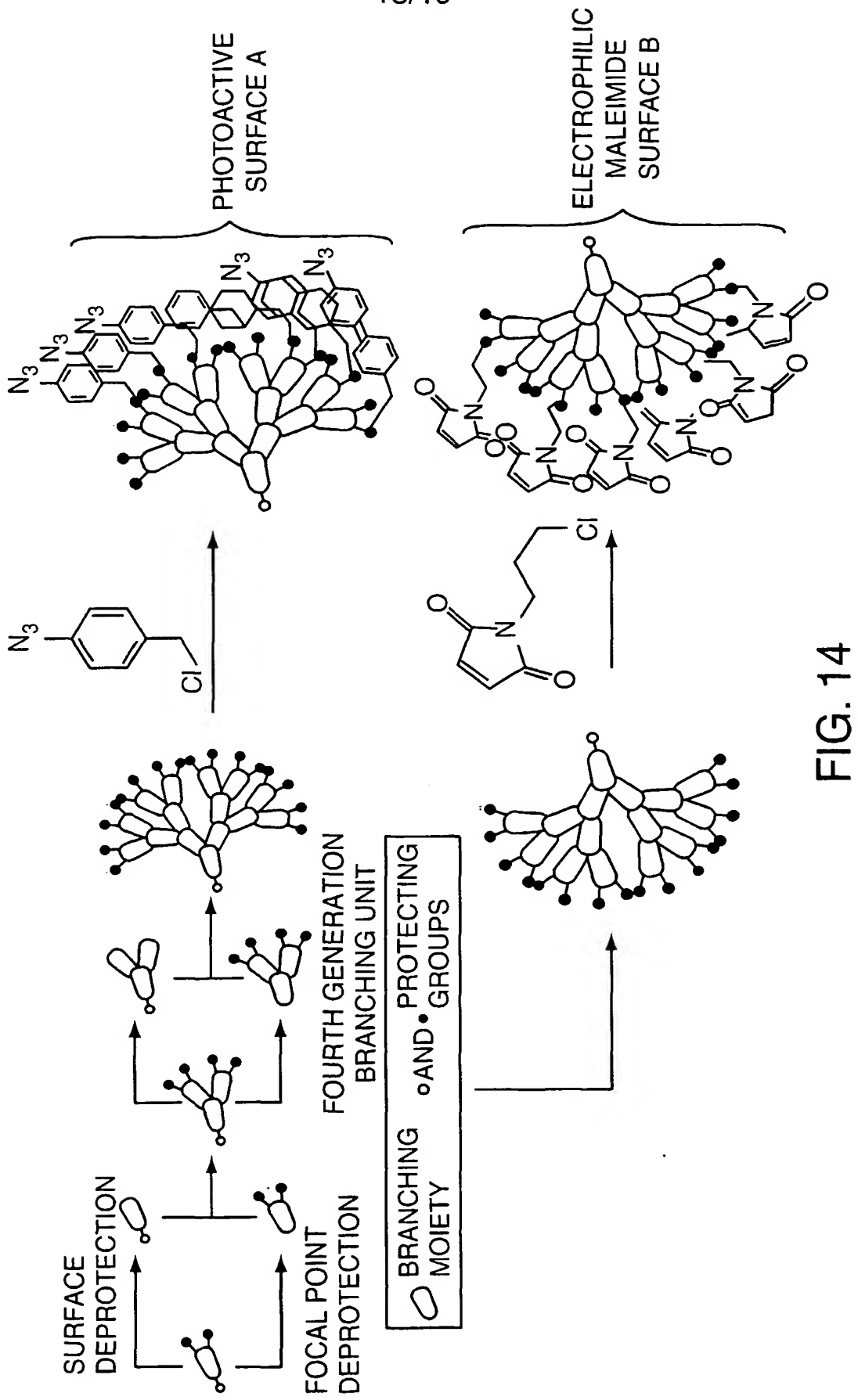

FIG. 14

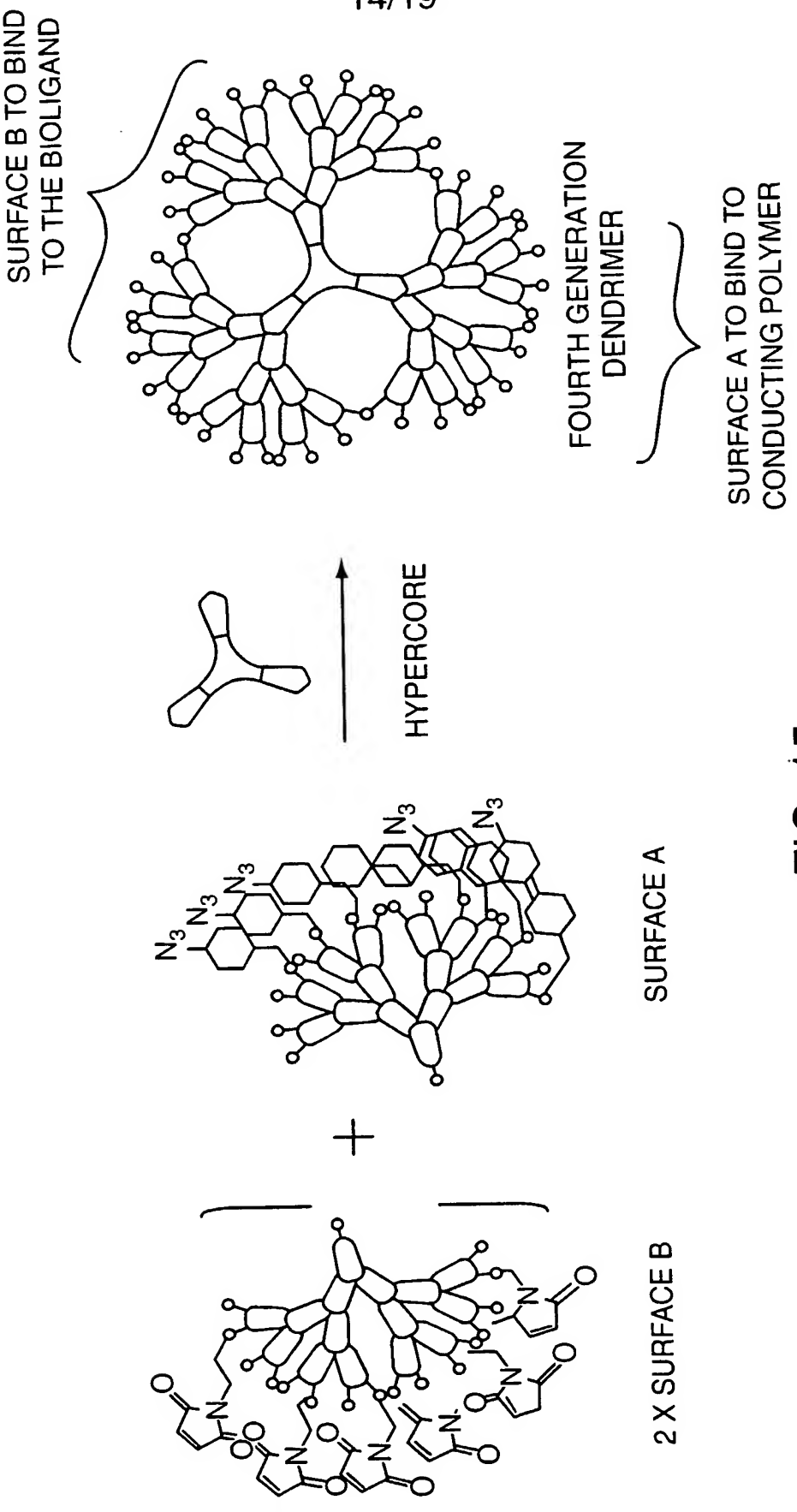

FIG. 15

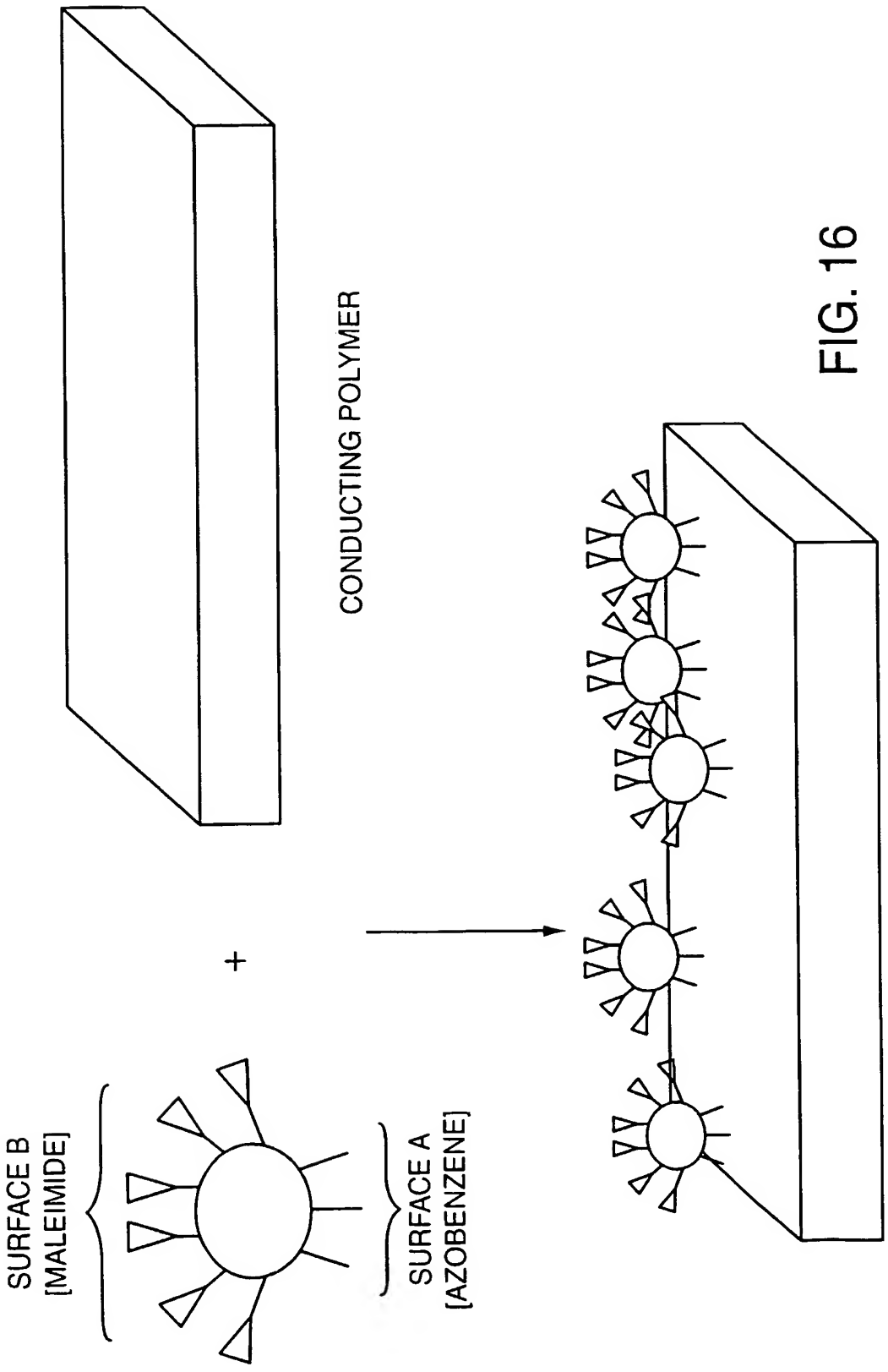

FIG. 16

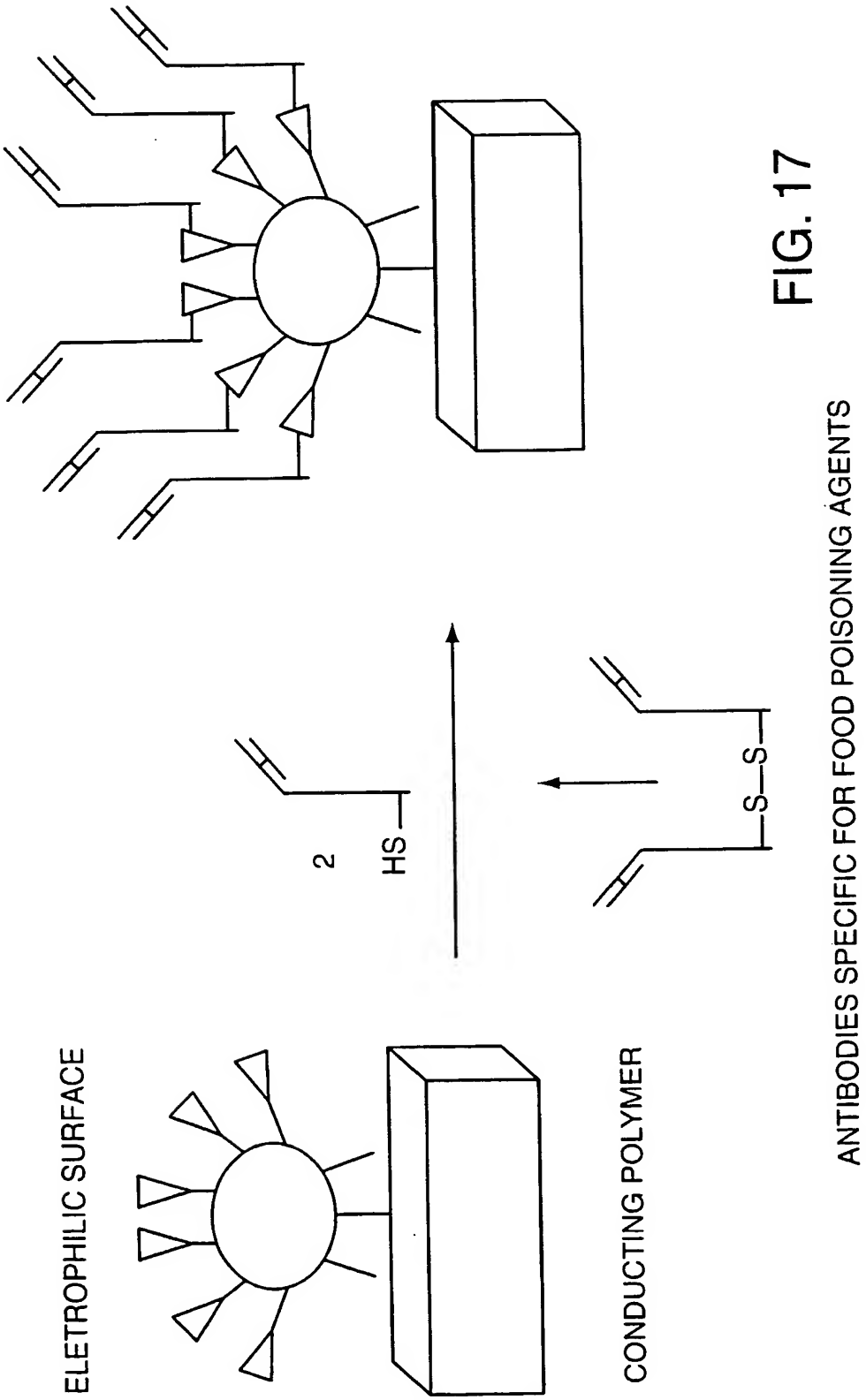

FIG. 17

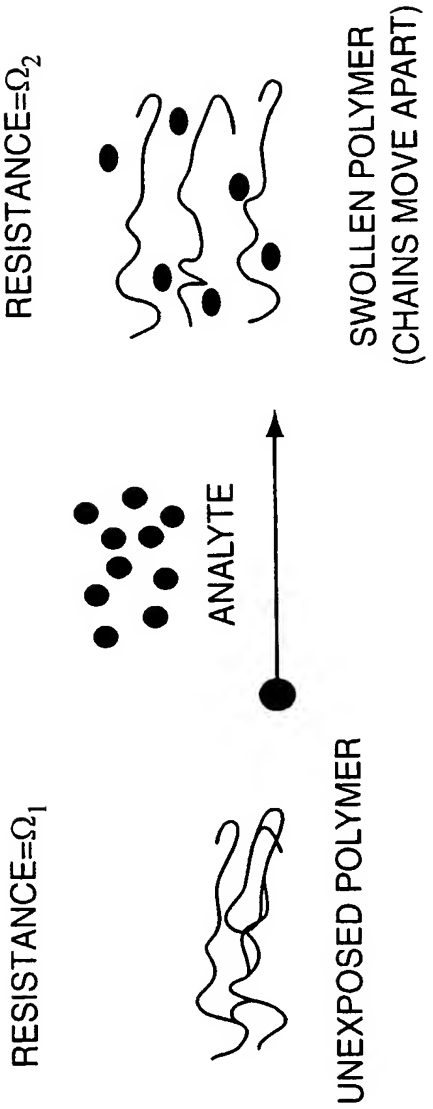

FIG. 18

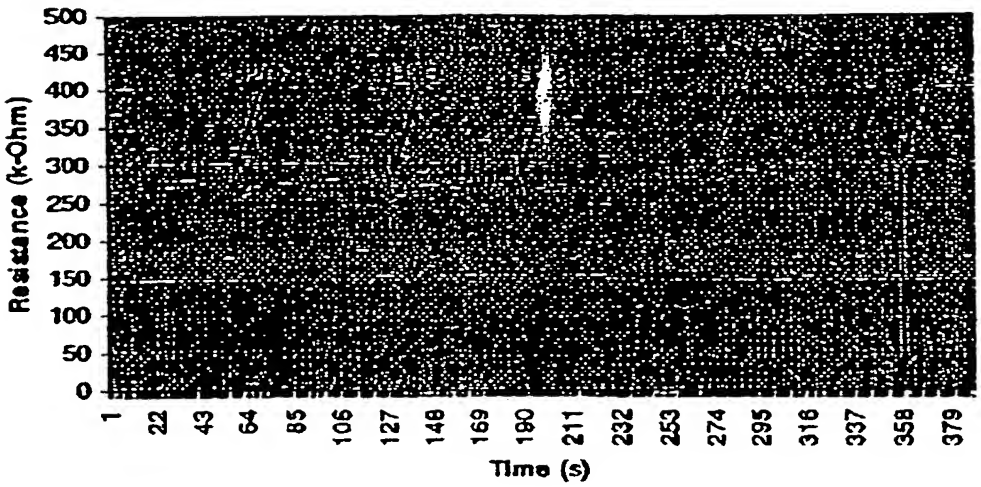

FIG. 19A

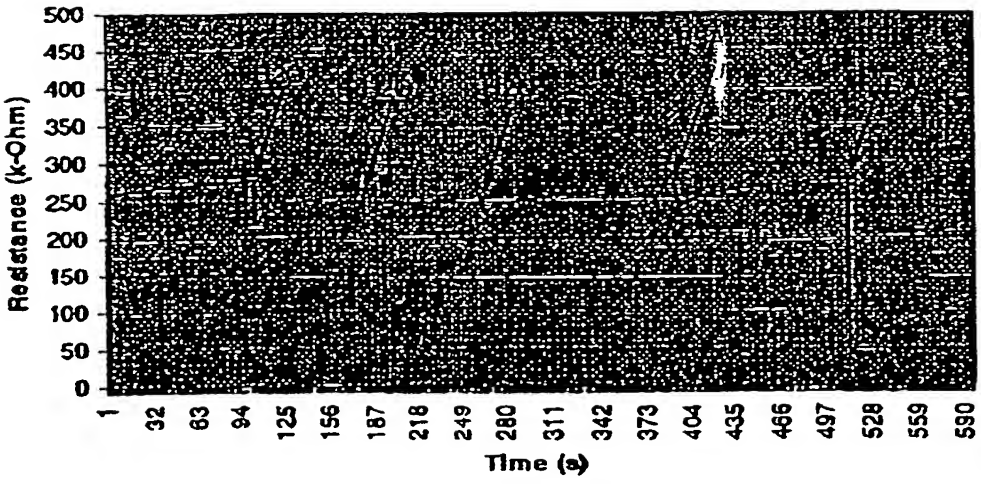

FIG. 19B

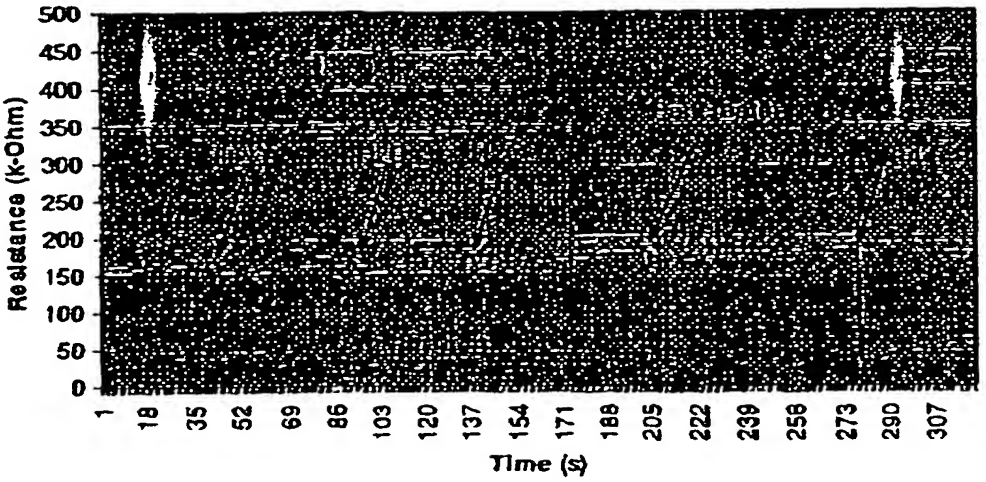

FIG. 19C

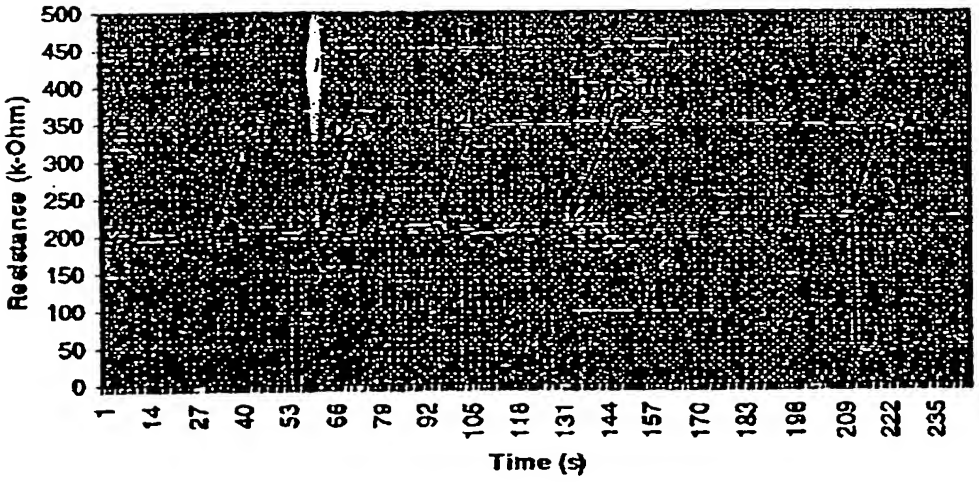

FIG. 19D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/31917

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :C12Q 1/00, 1/68, 1/34, 1/02; G01N 33/53; C12M 1/34
US CL :435/4, 6, 7.1, 18, 29, 287.1, 287.2, 811
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/4, 6, 7.1, 18, 29, 287.1, 287.2, 811

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4,444,892 A (MALMROS) 24 April 1984, see entire document.	1-79
Y	US 5,766,934 A (GUISEPPI-ELIE) 16 June 1998, see entire document, especially column 6, lines 4-10; column 23, lines 44-47 and column 25, lines 9-12.	1-79
Y	US 5,352,574 A (GUISEPPI-ELIE) 04 October 1994, see entire document, especially column 9, lines 60-65.	1-79
Y	US 4,716,122 A (SCHEEFERS) 29 December 1987, see entire document, especially column 2, lines 49-68.	13-30, 73
Y	US 5,788,989 A (JANSEN et al) 04 August 1998, see entire document.	31-37, 73

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 27 FEBRUARY 2002	Date of mailing of the international search report 09 APR 2002
Facsimile No. (703) 305-3230	Authorized officer AND Telephone No. DAVID M. NAFF (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/31917

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST
search terms: semi-conductive, sensor, polymer, polyfunctional, electrode, analyte, chamber, bifunctional, receptor, photoactivatable, linker, dendrimer